Immunological Study of Effectiveness of MNRI® Program on Respiratory Diseases

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This article presents an evaluation of the immunological effectiveness of the MNRI® program for patients with respiratory disease (see article by S. Masgutova, N. Akhmatova, I. Shubina, M. Kiselevskij, 2009). *Immunological Assessment of Effectiveness of Reflex Integration Therapeutic Program for Chronic Inflammatory Respiratory Disease. Immunology Journal*. Moscow, Russia. (Translation at www.MasgutovaMethod.com).

Keywords: Masgutova neurosensorimotor reflex integration, MNRI, recurrent obstructive bronchitis, lymphocytes, cytokines, natural killer cells

Introduction

Chronic inflammatory disease of the respiratory system and recurrent exacerbations are mainly caused by damage to the immune regulation mechanisms. Over the last years an increase in the incidence rate of this disease, with concomitant obstructive bronchitis syndrome, has been registered and the total percentage of inflammatory respiratory diseases continues to grow. Also, the frequency of cases requiring hospitalization has increased (Martinez, Curtis, Albert, 2008).

In this study treatment of chronic inflammatory diseases of the respiratory tract in children and adults was carried out by combining a complementary therapy using MNRI®, based on activation of the primary motor system in addition to the traditional treatment. Parameters of the immune status and cortisol level in children suffering from recurrent obstructive bronchitis (ROB) were studied to assist efficiency of the MNRI® therapy.

The study showed that combining MNRI® with conventional treatment corrects some disorders of the immune system mechanisms normalizing the number of lymphocytes (CD3, CD4, CD8) and NK-cells, the metabolic function of leukocytes, and the level of regulatory and anti-inflammatory cytokines. The therapy based on reflex patterns integration, improves the insufficient effects of standard therapy on immune system cells and strengthens polarization of the immune response of Th-1 type. These results from including MNRI® therapy included a decrease in the incidence of viral inflammatory diseases, positive healing dynamics, and pro-

longed remission in chronic respiratory diseases.

Materials and Methods

The study included the examination data of children and adults with chronic inflammatory and atopic diseases (chronic bronchitis, bronchial asthma) with concomitant obstructive bronchitis syndrome.

Observation cohorts involved 196 children from 2 to 13 years and 94 adults 20 to 60 years, who first underwent in-patient and out-patient treatment in clinical hospitals in Poland and Russia, then underwent MNRI®. Diagnosis was made in the hospital on the basis of complaints, objective examination, laboratory and x-ray analysis, according to classification of clinical forms of bronchitis and lung diseases of children and adults. The results were submitted by parents and patients on a voluntary basis and they signed contracts to use their personal data for research purposes. A control children's cohort included children of the same age group (examination of these children was performed according to parents' consent). Control for the immune-modulating effect of the MNRI® program was a children's group (15 children) suffering from ROB and receiving conventional treatment only.

The main parameters of the immune system were studied on the documentation of children's history of disease prior to therapy (initial background, group 2), after completing conventional therapy (group 3), and MNRI® program (group 4). Blood tests from patients of groups 3 and 4 were taken on the same day (last day of MNRI® therapy).

Neutrophil phagocytosis activity was evaluated by the following criteria: 1) percentage of phagocyting cells (% P); 2) absolute number of phagocytes; 3) phagocytic number (PN), average number of intracellular latex particles (the result of division of total phagocuted particles by cell number involved in phagocytosis); 4) phagocytic index (PI), the number of latex particles counted for one 'true' phagocyte. Latex particle size was of 1.3– 1.5 mm.

Flow Cytometry Analysis (FACS-Analysis) and Antibodies

Subpopulations of lymphocytes were determined by flow cytometry and monoclonal antibodies (mAb) (Caltag Laboratories, USA) against corresponding antigens. The cells were washed by cold phosphate-salt buffer (PBS) and stained by FITC and PE conjugated antibodies according to the manufacturer's instruction. The suspension was washed twice by cold PBS. The results were measured by flow cytometer FacsCalibur (Becton Dickinson, USA). The gate of cell population was fixed by front and side light scattering and cell size. The results counted 5000 cells in the gate. Statistics were calculated by using WINMDI 2.8 software.

Cortisol level (nMl/L) was determined in blood plasma by IFA with «Cortisol Kit», DPC «Immule », USA.

Statistical data evaluation was made by Student's t-criterion, Vilkokson's criterion with standard statistical program Windows (StatSoft 6.0).

Results and Discussion

Clinical assessment of the effectiveness of MNRI® therapy was performed in patients with chronic bronchitis (CB) in less than one year before treatment. MNRI® therapy of patients was started immediately or within one or two weeks following completion of basic therapy (antibiotics). Besides the patient group receiving alternative therapy (22 patients), a control group was formed including the same number of patients and the same disease severity, who received conventional therapy only.

MNRI® therapy resulted in a significant decrease of the period and severity of the disease. Frequency and duration of CB exacerbations in the study group (MNRI®) was substantially lower than in the control; no exacerbations were registered in 15 patients during one year after the MNRI® course, while three patients of the control group developed exacerbations. Before MNRI® therapy, 13 patients developed three or more exacerbations a year, and after a year of the MNRI® study group there was only one patient. But there were nine patients in the control group receiving standard therapy who developed exacerbations. The index of average days of sick leave registered during a year before and after the conducted therapy, decreased from 65.1±3.4 to 6.6±3.24 in the studied group (MNRI®) and from 55.8±2.53 to 46.8±2.58 in the control group (standard therapy), i.e. in 9.7 and 1.2 times, respectively.

The study of the effectiveness of MNRI® therapy also focused on bronchial asthma (BA). Development of bronchial asthma is closely connected with infection and sensitivity to bacterial antigens. These factors play the leading role in pathogenesis of not only infection-dependent bronchial asthma, but also a number of its

combined and transitive forms. This was the basis for the use of MNRI® therapy for patients with infection-dependent and mixed bronchial asthma. Clinical effectiveness of the therapy was assessed during a period of not less than 1 year. A significant effect was registered in the patients of this group in a reduced number and severity of recurrent disease, decrease in the use of antibiotics and hormones. A positive effect in 19 out 25 patients with BA and two patients demonstrated no marked effect (see article). Twelve patients of 25 of this group had a severe course of the disease. After MNRI® therapy completion the course of the disease became easier. A positive effect in the control group was registered in only 16% cases versus 76% in the MNRI® group.

The effectiveness of MNRI® therapy was studied in 35 children with BA aged 2–13 years. The therapy was performed immediately or within 1-2 weeks following the basic treatment approved in the Allergology Department (Kuvatov RCH, Ufa, Russia). MNRI® therapy effectiveness was assessed within 12 months after the last treatment. The results demonstrate positive effects of this alternative therapy on the mixed form of bronchial asthma in children. After the MNRI® course most children did not have any disease recurrences during 3 months, the rest of the patients also had some attacks with decreased frequency, duration, and severity of the disease. The disease attacks occurring in the later follow-up period were less frequent, and not as long and severe, as compared to those in the period before MNRI® therapy. Positive effects of the therapy were registered in 85.7% patients (versus 34.3% in the control group).

Along with the positive dynamics in the course of bronchial asthma, a 3.7-fold decrease in ARVD (acute respiratory virus infection) and 1.8- fold decrease in bronchitis incidence were registered after MNRI® therapy and consequently the number of antibiotics taken was reduced as well.

The following data present examination results of 10 children, aged 3–6 years suffering from ROB with the background of acute respiratory virus infection. At the moment of examination these children did not have sufficient basis for bronchial asthma diagnosis.

Analysis of the disease etiology showed that 94% of obstruction recurrences were induced by ARVI, which in 72% was complicated by bacterial infections. The tests in children with ROB showed a statistical significant decrease in the level of phagocyting neutrophils (% P) and their absorbing activity. At the same time a reliable increase of phagocytosis indexes was noted after MNRI® therapy in comparison with standard therapy. The children with ROB showed a decrease in bactericidal activity of leukocytes as a result of inhibition of oxygen-dependent microbicidal mechanisms. Leukocyte stimulation also decreased as compared to that of healthy children indicating a inhibition of immune system reactivity. The leukocyte index was reverting to normal values after the completed therapies.

A study of the subpopulation structure in children with ROB revealed the decreased number of D3+, CD4+, D8+, NK, NKT- cells. This data also indicates an increased number of cells expressing molecules of early (CD25) and late (HLA-DR) activation. An increase in B-lymphocyte and T-regulatory numbers was noted. After completing standard therapy, cell phenotype began to normalize gradually though an enhanced immune system reactivity was still noted, which disappeared after MNRI® therapy. The levels of CD3, CD4, CD8, CD25 lymphocytes and NK-cells were already reliably registered in groups 3 and 4.

Cortisol levels in the morning and evening blood plasma samples of children with ROB was statistically significantly lower than that of the control group. Conducting immunocorrecting treatment involving both standard and MNRI® therapy led to a gradual shift towards increased cortisol levels, however the cortisol level of the morning sample after standard therapy was consistently lower than that of the sample taken after adding the MNRI® therapy. It is well-known that the anti-inflammatory and immunomodulating effect of cortisol in a physiological concentration is associated with lipocortine synthesis, which blocks formation of lipooxi- and ciclooxigenases products of arachidonic acid. Lipocortine phosphorilated form promotes intensive synthesis of IgE – a suppressive factor and therefore inhibits IgE synthesis. The pathological chemical phase of ROB leads to an extensive release of LTB4 that plays a leading role in formation of bronchus obstruction (Sechenov, 1935). Possibly the initially low cortisol concentration in this disease may inefficiently regulate formation of LTB4, IgE and other effectors, thus potentiating Th2 response.

This fact is confirmed by the decrease in the production rate of pro-inflammatory cytokine IL-1, regulatory IFN- and IL-12, and the enhanced rate of antiinflammatory cytokines IL-4, IL-10 in peripheral blood mononuclear leukocyte culture (PBML) in children with ROB. In spite of the positive dynamics of cytokine levels after standard therapy, their concentration in the culture medium of leukocytes was reliably different from the control

group. When MNRI® therapy was added, the level of regulatory and anti-inflammatory cytokines normalized.

Thus, children with ROB in the exacerbation phase provoked by viral infection develop failure in immunological reaction. Significant changes were noted in innate (inhibition of phagocytic neutrophil function, decrease in the number of NK and NKT-cells) and adaptive immunity (decrease of T and B-lymphocyte number), levels of pro-inflammatory (IL–1) and regulatory (IFN, IL-12) cytokines. The results suggest that the immune response in children with this disease is polarized towards the Th2 pathway, since IL-4 products are Th2-lymphocytes, while IL-12 polarizes immune response towards the Th2 pathway.

The low cortisol level did not allow adequate neuroendocrine system response to the pathogen. Standard therapy had practically no effect on the number of D3, CD4, and NK and T-regulatory cells. A tendency to immunophenotype normalization was noted when additional MNRI® therapy was performed. Standard treatment exacerbated depression of parameters of neutrophil phagocytosis, pro-inflammatory IL- production and regulatory cytokines, and had no effect on cortisol level.

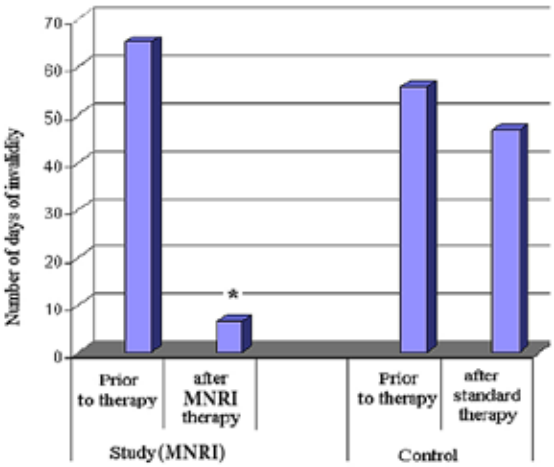

Figure 1. Effect of MNRI® therapy in CB of adult patients.*-<0.01 in comparison with control group (prior to therapy)

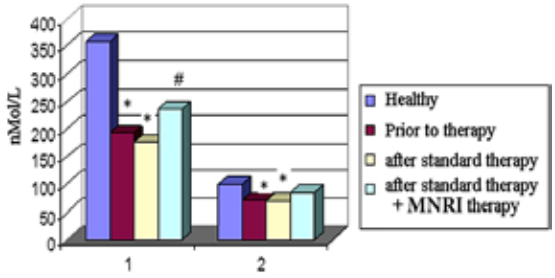

Figure 2. A level of cortisol in plasma of blood at children with ROB. 1 - morning test, 2 - evening test. *- <0.01 in comparison with control group (prior to therapy); # - P <0.01 in comparison with group after standard therapy.

Therefore, after conventional therapy despite clinical improvement, children with ROB still had insufficient cortisol symptoms and simultaneous inhibition of T-cell immunity.

After the addition of MNRI® to the standard therapy of ROB the results showed a reliable increase in the absolute number, of segmented neutrophils, both in relation to the initial number and to the standard therapy in the group number. MNRI® with standard drug therapy leads to an increase in the absorbing activity of neutrophils and normalization of leukocyte metabolic function, raising the stimulation index of NST-test to the normal value (from 0.97 to 1.3). In addition, a statistically significant increase of the number of cells expressing differentiation antigens and NK-cells (CD16) was registered. NK-cells are the key effectors of innate immunity and play an important biological role in mechanisms of immune surveillance targeting tumor cells; in destruction of the virus and parasite infected cells, in regulation and differentiation of bone marrow cells (eliminating rapidly proliferating hemopoetic = hematopoetic cells); and in graft-versus-host reaction.

In addition to its immunoregulating effect, the therapeutic MNRI® program increased cortisol levels up to physiological concentrations. IL-1 produced by mononuclear leukocytes enhanced the secretion of glucocorticoids as a consequence. MNRI® therapy may determine adrenal cortex regulation by hypophysis ACTH by IL-1 production. Thus MNRI® therapy enhanced the Th-1 pathway immune response and its positive effect on immune system cells surpassed that of standard therapy.

Follow-up observation for one year showed that ARVI incidence in children receiving MNRI® decreased by 2.8 times (from 4.51±1.1 to 1.6±0.62) and the frequency of exacerbation decreased by 2.5 times (from 6.4±2.35 to 2.52±0.33). ARVI incidence and exacerbation frequency in children receiving standard treatment only remained at the previous rate. Similar results were noted in patients with CB and BA after addition of MNRI® to the basic therapy the disability index in children decreased, the frequency and severity of the disease reduced, and, ARVI and bronchitis incidence decreased.

We can conclude that including the MNRI® therapeutic program in treatment of children with recurring obstructive bronchitis and adults with BA and CB leads to correction of the impaired mechanisms of the immune

system. MNRI® therapy leads to a decreased incidence of ARVI disease, of ARVI disease incidence, an increase in the positive dynamics of the course of chronic respiratory diseases and an extension in the remission of the disease.

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Dear kids, we wish you a strong immune system and good growth! We scientists wish you all good health, less germs and viruses, and a strong immune system! We also thank all the adults who helped us in this research and wish them good health, less germs and viruses, and a strong, active, and healthy lifestyle to keep their immune system activated! – Authors