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NEUROGENIC INFLAMMATION INDICES IN CHILDREN OF PRIMARY SCHOOL AGE WITH RECURRENT UPPER RESPIRATORY TRACT INFECTION.

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Abstract

The children of primary school are prone to frequent respiratory diseases. In the structure of general morbidity, this pathology ranks first. Their prevalence is more than 60%, which leads to frequent school absences, lack of active games and outdoor walks, in view of this the child's time increases over the means of mass electronic communication and electronic games, leading to high adherence, up to and including to addiction, forming in the child hypodynamia and increased psycho-emotional stress, which together has a negative impact on the functioning of the regulatory systems of the child's body. Recurrent upper respiratory tract infection contributes to the tension of neuro-endocrine-immune regulation, leading to an imbalance of neuropeptides - mediators of neurogenic inflammation. The aim of this work is to study the indices of neurogenic inflammation in children of primary school with recurrent upper respiratory tract infection. We examined 130 children with upper respiratory tract infection at the age of 6 to 9 years in the period of somatic well-being. The serum content of vasoactive intestinal peptide, substance P and NO final stable metabolites in children was analyzed. An increase of serum substance P in of schoolchildren with upper respiratory tract infection was found, which had statistically significant differences with the indices of healthy children ($p < 0.05$). It has been proved that in children with upper respiratory tract infection due to an increase in serum NO final stable metabolites, which have a pro-inflammatory orientation. It was found a significant decrease of the serum vasoactive intestinal peptide content in children with upper respiratory tract infection. Children with upper respiratory tract infection upper respiratory tract infection in the period of somatic well-being have an increase in the concentration of serum substance P and final stable metabolites NO. In the period of somatic well-being children of primary school with upper respiratory tract infection have a decrease of the vasoactive intestinal peptide serum level.

Keywords: *neuropeptides, inflammation, children, recurrent upper respiratory tract infection.*

Introduction

In most countries of the world, respiratory diseases in childhood rank first in the structure of the overall incidence, their prevalence is more than 60% in children and 50% in adolescents, and the increment is 5-7% per year [*Chandrasekharan B, et al., 2013*]. The prevalence of respiratory pathology among the child population was 67.11% in Ukraine within 2016, [*White CM, et al., 2010*]. Therefore, upper respiratory tract infection (URTI) are a significant problem in the practice of a pediatrician doctor, and a family medicine doctor, especially in cases of their frequent recurrence i.e. a recurrent course. In the group of patients with recurrent URTI include children aged 6 to 7 years who sick 4 or more times per year [*Shamrai IV., 2013*].

At the same time, nowadays in schoolchildren, in addition to significantly expanding contact with respiratory pathogens, the information field is additionally intensively influenced: on the one hand, the means of mass communications with electronic games, on the other, an intensive educational program and additional elective classes. This leads to the stress on neuro-immune homeostasis, and negatively affects the frequency and course of respiratory tract

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diseases [*Ang S-F, et al., 2011, Malhotra R., 2016*]. In combination, these factors cause the formation of hypodynamia and increased psycho-emotional stress, which have a negative impact on the functioning of the regulatory systems of the child body, which in the future can lead to serious diseases of the cardiovascular system and death [*Mukvich OM, et al., 2013, Goldfield GS, et al. 2011*].

Control over the functioning of adaptive homeostasis belongs to the nervous and endocrine systems. The effect of the nervous system is realized in the tissues through the cholinergic and adrenergic receptors of the cells by changing the activity of the endocrine glands whose regulation centers are in the hypothalamus, and also by the formation of active protein molecules – neuropeptides [*Shafranssky VV, 2015*]. In total, 18 families of these substances are known, the most important of them are opiates, neurohypophasic, tachykinins etc.

It is known that the complex pathogenesis of the inflammatory reaction goes beyond the framework of only immune mechanisms, involving neuronal interactions. This is due to the ability of cells of the immune system, stimulated in inflammation, to produce neuropeptides similar to those produced in the central nervous system (CNS) [*Ganea D, et al., 2015*].

In recent years, it has been proven that the brain, in addition to the most complex mental and neurological functions, not only takes part in the generation and regulation of the immune response in the central nervous system, but it itself is one of the central organs of the immune system. A similarity in the organization and functioning of neurons of the brain and cells of the immune system is established. Neuroendocrine cells of the brain and cells of the immune system act in close cooperation and show signs of similarity in their organization [*Mukvich OM, et al., 2013, Shafranssky VV, 2015*].

Among neuropeptides, anti-inflammatory effects such as the vasoactive intestinal peptide (VIP) and pro-inflammatory effects-substance can be distinguished [*Grigoryeva VA, et al., 2011, Ovcharenko LS,* et *al., 2015, Rechkina EA, 2013*]. VIP is produced by neurons, endocrine and immune cells and is present in most organs, including the central nervous system, heart, lungs, thyroid gland, kidney, urinary and gastrointestinal tract, genital organs and immune system [*Kazyukova TV et al., 2012, Uryasev OM, Shakhanov AV, 2017*]. VIP suppresses the expression of other inflammatory mediators [*Ovcharenko LS, et al., 2015*]. Recent studies have shown that VIP acts directly on the immune system of the mucosa, where it increases the synthesis of IgA, which indicates the critical role of VIP in inducing oral tolerance [*Ovcharenko LS, et al., 2015*].

Neurogenic inflammation includes a number of inflammatory reactions caused by the activation of primary sensory neurons and the subsequent release of inflammatory neuropeptides [*Albickij VJu, et al., 2006*]. Proinflammatory peptides are expressed mainly in neuronal and glial cells of human central and peripheral nervous system. These peptides act primarily as neurotransmitters in the CNS and as mediators of non-noradrenergic, non-cholinergic transmission of stimulating neurotransmitters in autonomic nerves. Among all proinflammatory peptides, substance P be better characterized. This was shown with the functional involvement of this neuropeptide in nociceptive (painful) reactions and neurogenic inflammation [*Ovcharenko LS, et al., 2013*].

Substance P is a peptide consisting of 11 amino acids, which regulates the immune balance on mucosal surfaces and in other foci of chronic inflammation [*Ganea D, et al., 2015*]. Substance P is currently considered as the main mediator of neurogenic inflammation, capable of causing such pathophysiological reactions as edema, hypersecretion of mucus, bronchospasm, decreased vascular tone, increased permeability of postcapillary venules, penetration of immune cells into tissues and secretion of glands [*Waschek JA, 2013*].

Being a neurotransmitter, substance P participates in many immune reactions. It is produced by macrophages, T cells, dendritic cells and eosinophils. The production of substance P by macrophages is induced by lipopolysaccharides of bacteria [*Waschek JA, 2013*]. Substance P acts as a pro-inflammatory mediator, enhancing monocyte / macrophage chemotaxis, monitors the T1 response, enhancing the production of IFN-γ [*Waschek JA, 2013*]. Being capable of inducing both T1 and T2 types, it stimulates the pro-inflammatory activity and secretion of TNF-α, IL-1, IL-6, IL-8, IL-12, IFN-γ, production of superoxide anion, enhancing the overall inflammatory response [*Albickij VJu, et*

al., 2006; *Goldfield GS, et al., 2011*].

Another substance with pro-inflammatory properties is nitric oxide (NO). NO is the ubiquitous and unique molecule of the biological messenger, which is biosynthesized from the amino acid of L-arginine. NO is produced inside endothelial cells, macrophages and neuronal tissues. NO mediates a wide range of important biological processes, such as endothelium-dependent vasodilatation, inhibition of platelet aggregation, inflammation, immunoregulation, and information transfer between neurons in the central nervous system and the peripheral nervous system [*Ovcharenko LS et al., 2013*; *Souza-Moreira L et al., 2011*; *Waschek JA 2013*]. It is known that NO has a pro-inflammatory effect and affects the immune system. The NO molecule is a simple radical and, due to its small size and lack of charge, NO easily passes through the cell membranes [*Ganea D et al., 2015*]. NO stimulates perivascular neurogenic inflammation by facilitating the synthesis and release of immunoreactive neurotransmitters such as substance P from nociceptive afferent fibers [*Ovcharenko LS et al., 2013*]. An increase in NO concentration in children is observed in conditions that are accompanied by a change in the activity of cytokines-acute infection, chronic bronchopulmonary system diseases, persistent intracellular infection. The concentration of NO in the exhaled air in children correlates with neutrophilia [*Ganea D et al., 2015*]. Its indirect cytotoxic effect of NO realizes through the action of free radicals – peroxynitrite, nitrogen dioxide, hydroxyl radical, which initiate lipid peroxidation. Metabolites NO - nitrates and nitrites play an important role in the development of inflammation [*Waschek JA, 2013*].

The role of neuropeptides in the immune response [*Ganea D et al., 2015*; *Waschek JA, 2013*] is important for determine the effect of these mechanisms on the state of the initial vegetative tone in children of primary school age with recurrent URTI. Possible disorders of neuroimmune mechanisms, as well as the consequences of hypodynamia, due to frequent exemptions from physical education, the prohibition of attending sports sections, in children with functional cardiovascular disorders are a risk factor leading to the formation of cardiovascular pathology [*Mukvich OM, Omelchenko LI, 2013*].

The aim: of this work is to study the indices of neurogenic inflammation in children of primary school with recurrent URTI.

Material and methods

During the observation, the 130 children aged 6 to 9 years were divided into three groups. Group 1 – children with functional disorders of the cardiovascular system and recurrent forms of respiratory tract diseases (n = 50); Group 2 – children with functional disorders of the cardiovascular system, who are ill episodically (n = 50); Group 3 – control group of conditionally healthy children (n = 30).

The content of serum VIP, substance P and the final stable metabolites of NO (FSM NO) in children of the observation groups was analyzed. The study of blood plasma contents of end-stable metabolites of nitric oxide (nitrates, nitrites) was carried out by restoring nitrates to nitrites with the determination of the latter by reaction with a Gris reagent. The optical length was measured on a spectrophotometer at a wavelength of 540 nm. Calculation of the number of nitrites was carried out on a calibration graph, built on nitrogen [*Green L. C. et al., 1982]*.

A quantitative determination of R and VIP substance in the blood serum was carried out by the immunoassay assay on a SUNRISE photometry analyzer ("TECAN Austria") using the ELISA test "Substance P" and "Vasoactive Intestinal Peptide" produced by "Peninsula Laboratories, LLC" (San Carlos, USA) [*Avrameas S, 1992, Porstmann T and Kiessig ST, 1992*].

The statistical analysis was carried out using the "Statistica 7.0" software package (Stat Soft Inc., 2004). The study uses statistical methods for processing the information obtained: Fisher's angular criterion, mean (M), the mean (m) error, the Student's t-test, the Mann-Whitney test. A ROC analysis of diagnostic tests was performed, based on calculating the predicted value of the area under the ROC curve (AUC) with the calculation of the cut-off point.

Results

A survey of children of early school age was conducted during the period of somatic well-being. The results of the study are shown in Table 1.

In determining the concentration levels of substance P, VIP, FSM NO in the serum of the children of the group 1 in the period of somatic well-being, there was a statistically significant (in comparison with the group 3) increase of serum substance P and VIP concentration ($p < 0.05$). At the same time,

Table 1.

Parameters of serum content of neurotransmitters in children of observation groups in the period of somatic well-being (M ± m)

Indicator (ng/ml)	Observation group		
	Group 1 (n=50)	Group 2 (n=50)	Group 3 (n=30)
Substance P	0.48±0.06 $^{\Delta}$	0.44±0.03	0.41±0.04
VIP	0.27±0.02 $^{*\Delta}$	0.34±0.02	0.38±0.03
FSM NO	21.23±1.11 $^{\Delta}$	20.3±1.17	17.67±1.04

Notes: * p <0.05 - when compared with the results of group 2; Δ p <0.05 - when compared with the results of group 3.

a decrease in the concentration of VIPs was noted and, in comparison with the group 2, p <0.05.

Data of the low values of the concentration of substance P in the observation groups, it became necessary to analyze the confidence intervals of this transmitter.

When comparing groups 1 and 2, there was no statistically significant difference in confidence intervals (Figure 1A). Analyzing the levels of the indicator of substance P in children with recurrent URTI and episodically ill we can conclude that there is no significant difference (U = 1071.0, p> 0.05), this is confirmed by the ROC analysis data (Figure 2A).

ROC analysis of the concentration distribution of substance P between the groups 1 and 2 showed that the cut-off point is the concentration of substance P - 0.31 *ng/ml*. The concentration of substance P above this indicator is associated with an increased risk of recurrent URTI. The optimal cut-off point was 0.31 *ng/ml* (area under the AUC 0.6 curve) with a sensitivity of 26.0% and a specificity of 90.0% (Figure 2A).

When comparing groups 1 and 3, a statistically significant difference in the values of confidence intervals was revealed. It also confirmed by the selection of children of observation groups in the period of somatic well-being. The revealed differences allow to say that among children with recurrent URTI and control group there is a statistically significant difference in the level of concentration of substance P (U = 529.5, p <0.05). These differences in results are confirmed by ROC analysis data (Figure 2B).

ROC analysis of the content distribution of substance P in the groups 1 and 3 showed that the cut-off point is a concentration of 0.3 *ng/ml* - concentration. The level of concentration of substance P above this indicator is associated with an increased risk of recurrent URTI occurrence. The optimal cut-off point is 0.3 *ng/ml* (area under the AUC 0.7 curve) with a sensitivity of 33.0% and a specificity of 100.0% (Figure 2B).

Estimating the received indices of the VIP neuropeptide, a comparison of observation groups 1 and 2 revealed a statistically significant difference in confidence intervals (Figure 1B).

Determination of the concentration of VIP in the serum in children of the group 1 and 2 showed significant differences (U = 473.5, p> 0.05). The same differences confirmed by the ROC analysis data (Figure 3A). For the concentration of VIP in the serum, a cut-off point corresponding to a level of 0.34 ng/ml was determined. Concentration below this level is associated with an increased risk of developing recurrent URTI (area under the AUC curve of 0.8) with a sensitivity of 68.0% and a specificity of 100.0%. Similar results were obtained by comparing the groups 1 and 3. A significant difference in the values (U = 373.0, p <0.05) was established, which confirmed by the confidence intervals. The differences obtained are also

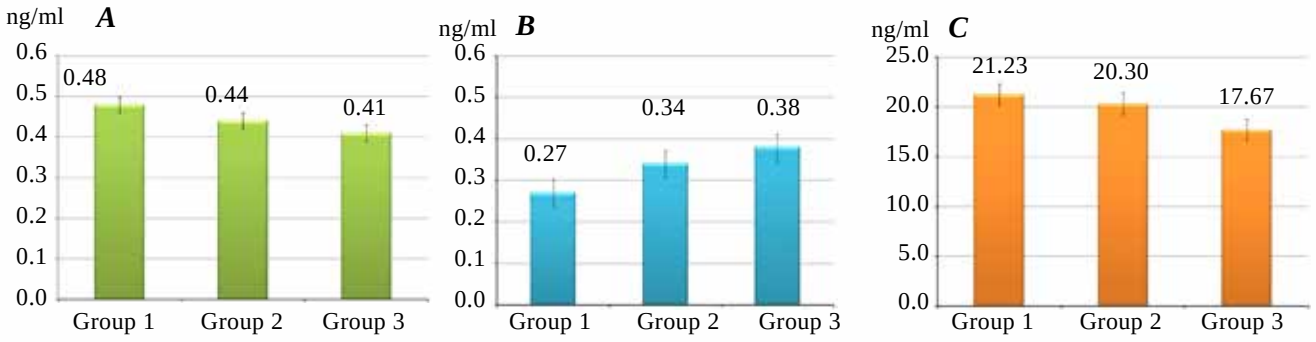

Figure 1. Confidence intervals for the concentration of the substance P (A), for the level of vasoactive intestinal peptide (B), and for the level of final stable metabolites of NO (C) in the observation groups.

confirmed by the ROC analysis data (Figure 3B). The cut-off point for VIP, the neuropeptide concentration was determined to be 0.28 ng/ml. Indicators VIP below this concentration will be associated with the risk of developing recurrent URTI (area under the AUC curve of 0.8) with a sensitivity of 100.0% and a specificity of 50.0%.

The determination of serum FSM NO in schoolchildren, depending on the distribution groups, showed a slight difference between the groups 1 and 2 (U = 873.0, p> 0.05) and significant differences between the groups 1 and 3 (U = 377.5, p <0.05). What is confirmed by confidence intervals (Figure 1C).

The ROC analysis determined the cut-off point

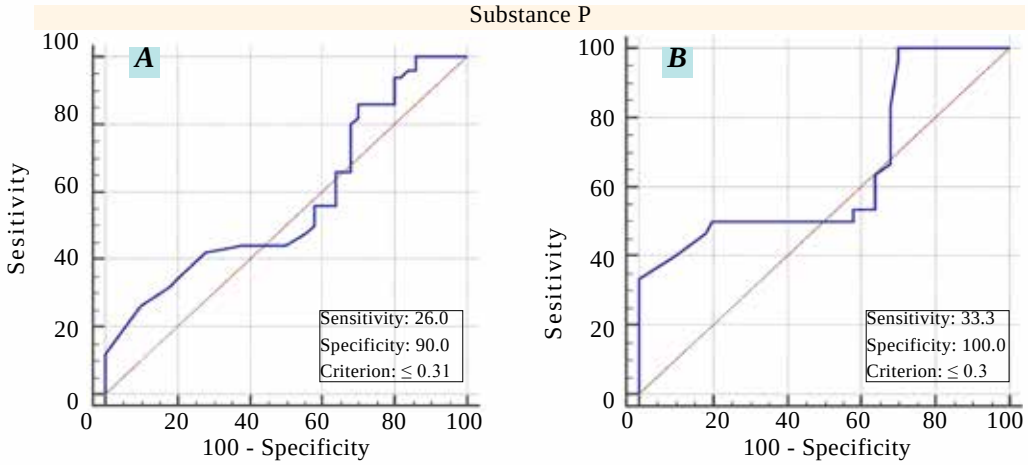

FIGURE 2. *Sensitivity and specificity of the cut-off point of serum substance P in children 1-2 groups (**A**) and 1-3 groups (**B**).*

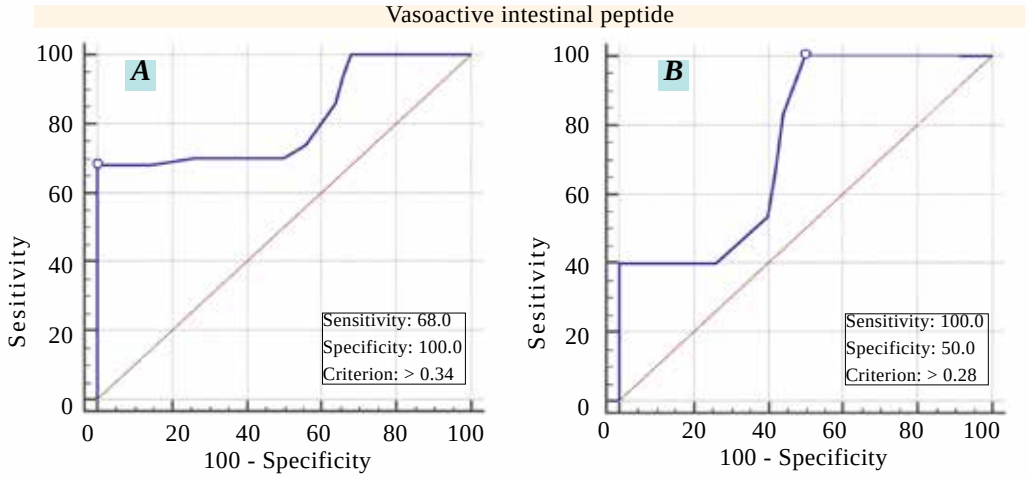

FIGURE 3. *Sensitivity and specificity of the cut-off point of serum VIP in children 1-2 groups (**A**) and 1-3 groups (**B**).*

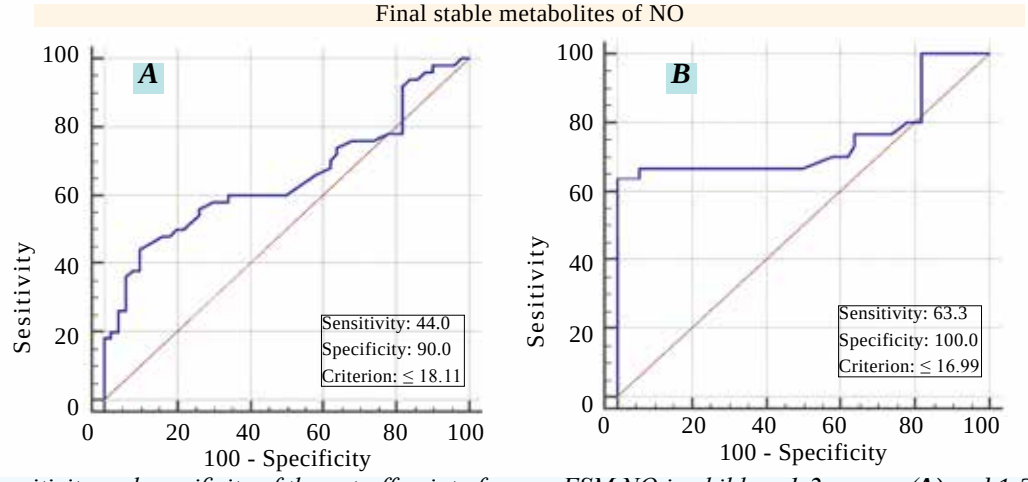

FIGURE 4. *Sensitivity and specificity of the cut-off point of serum FSM NO in children 1-2 groups (**A**) and 1-3 groups (**B**).*

for FSM NO in comparison groups 1 and 2 to 18.11 *ng/ml*. The concentration of FSM NO above this index was associated with an increased risk of recurrent URTI (area under the AUC 0.7 curve) with a sensitivity of 44.0% and a specificity of 90.0% (Figure 4A). When comparing the indices in the groups 1 and 3, the cut-off point was the concentration of FSM NO – 16.99 *ng/ml*. Exceeding this concentration is associated with a high risk of recurrent URTI formation (area under the AUC 0.75 curve) with a sensitivity of 63.0% and a specificity of 100.0% (Figure 4B).

Discussion

Currently, modern schoolchildren are under the influence of an intense information field on the one hand, and under the influence of infectious pathogens on the other hand, as a result, among this population of children, a group with recurrent URTI is allocated.

Because the complex pathogenesis of the inflammatory reaction goes beyond only immune mechanisms, neuronal interactions are involved in the inflammation process. This is due to the ability of immune-competent cells, stimulated in inflammation, to produce neuropeptides similar to those produced in the central nervous system [*Grigoryeva VA et al., 2011*].

In the human body, neuropeptides that exert anti-inflammatory effects are isolated, one of them is the VIP, and pro-inflammatory effects such as substance P, and also to pro-inflammatory neurotransmitters include NO [*Ovcharenko LS. et al., 2015*; *White CM et al., 2010*; *Shafranssky VV., 2016*].

On the basis of the modern theory of neuro-endocrine-immune regulation, immune reactions can be considered as components of neuroendocrine activity, and the immune response itself is impossible without the friendly participation of the nervous and endocrine systems. In the implementation of neuroimmune interactions, the humoral regulatory factors that affect both these systems are involved. [*Uryasev OM, Shakhanov AV, 2017*].

Frequent repeated URTI in children of primary school age adversely affect the functional state of respiratory, cardiovascular, CNS, changing the mechanism of development of the pathological process, which is clinically manifested by late recovery. The need to study the levels of neurotransmitters in the serum of schoolchildren with recurrent URTI allowed to determine the degree of involvement of neuro-immune inflammation in the pathogenesis of the disease and to identify a risk group for the development of recurrent respiratory tract diseases.

Comparative analysis of the content of substance P in the schoolchildren serum showed significant differences between the group 1 and group 3 ($p < 0.05$). Exceeding the established concentration of substance P increases the risk of developing recurrent diseases.

The pro-inflammatory focus of FSM NO especially in children of the group 1 can enhance the activity of neurogenic inflammation. Probably, this is due to the fact that in children with recurrent URTI against the background of increased activity of immune-competent cells as a result of prolonged antigenic (viral-bacterial) stimulation production of CNS neurotransmitters increased in response to the stressful situation associated with recurrent diseases [*Uryasev OM, Shakhanov AV, 2017*].

Excess of the established concentration of FSM NO increases the risk of developing recurrent diseases. A significant decrease in the content of anti-inflammatory transmitters, in particular VIPs in children in the group with recurrent URTI, is not able to inhibit the activity of neurogenic inflammation.

Conclusion

In children of early school age with recurrent URTI in the period of somatic well-being there is an increase of neurogenic inflammation mediators - substance P and FSM NO in serum concentration. The period of somatic well-being in children of early school age with recurrent URTI is characterized by a decrease in the level of anti-inflammatory neuropeptide VIP concentration. The use of ROC analysis is a mechanism for predicting the formation of risks for the development of recurrent respiratory tract diseases.

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