

Results of the efficiency study of the “Normogen”  
immunobiological preparation application to oncology patients  
by the data of immune status investigation.

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Recently, in the practice of oncology, immunobiological preparations on the basis of specific antibodies are more and more commonly used. High selectivity of such preparations makes oncology patients' treatment more safe than treatment with chemotherapeutical medicinal agents.

But introduction of alien proteins into organism of a recipient has noticeable affect on the immunological parameters of the patient's organism. In this aspect, the study of the influence of domestically designed antitumor "Normogen" preparation on the immunological reactivity parameters of oncology patients is of interest.

Complex immunological investigation was carried out on the base of the laboratory of clinical immunology of the Central Clinical Hospital of the Medical Center of RK President's affairs management

Estimation of the "Normogen" preparation efficiency<sup>1</sup> was carried out on the ground of the dynamics of the immune status parameters and hematological parameters.

The control immunology investigation was realized in a month after the fulfilled treatment.

A comparative analysis of hematological parameters of oncological patients that were studied before and after the treatment (basic group 1 and 2), and also of the control group people (group 3 – practically healthy persons), showed that the changes in such parameters as the content of the leucocytes in 1 liter of blood, and relative number of neutrophils and lymphocytes were statistically significant (table 1).

Table 1

**Hematological parameters of oncological patients that were studied before and after the treatment with the "Normogen" preparation introduction (X±m)**

Parameter	Basic group before the treatment (gr.1)	Basic group after the treatment (gr.2)	Control group (healthy, gr-3)
Hemoglobin	135,07±36,1	131,37±36,44	145,87±38,98
Erithrocytes	4,64±1,24	4,65±1,29	4,7±1,26
Average content of Hb in an erythrocyte	29,99±8,66	29,15±8,42	31,33±8,37
Color parameter	0,91±0,26	0,87±0,24	0,97±0,26
Hematocrit	40,41±11,66	40,47±11,68	47,27±12,63
Average volume of an erythrocyte	90,77±26,2	88,73±25,61	97,28±24,4
Trombocytes	309,92±82,83	258,49±71,69	265,15±70,86
Leucocytes	6,84±1,83	26,67±7,4***	6,82±1,82
Segmented neutrophils	64,76±17,31	70,99±19,69**	62,81±16,79
Eosinophils	3,86±1,29	3,14±1,11	4,03±1,08
Basocytes	8,56±3,5	6,02±3,48**	0,65±0,17
Monocytes	8,17±2,58	8,06±2,33	3,91±1,05
Lymphocytes	28,14±7,52	23,84±6,61*	33,13±8,85
ESR	30,04±8,03	33,97±9,42	9,09±2,43

Comments - \* - p <0,02; \*\* - p <0,01; \*\*\* - p <0,001;

As it can be seen from the table 1, the "Normogen" preparation has the most

stimulating influence on such parameter as the content of the leucocytes in 1 liter of blood.

While the number of leucocytes in the oncological patients were  $6,84 \pm 1,83 \times 10^9$ /liter before the treatment, this parameter increased with the high degree of reliability ( $p < 0,001$ ) to  $26,67 \pm 7,4 \times 10^9$ /liter in a month after the immunocorrection with the "Normogen" preparation.

This rise has a disharmonic character, because it highly exceeded such parameter of the control group of people that was  $6,82 \pm 1,82 \times 10^9$  /liter ( $p < 0,001$ ). Such significant exceeding of the parameter can be explained by high rate of tumor cell destruction caused by the action of the "Normogen" preparation and subsequent intoxication of the organism. Occurrences of intoxication manifested in weakness, nausea, rarely vomiting, rise of body temperature to 38 °C.

Though the rise of the leucocytes content, detected at the treatment, was extremely excessive, in this case a leucocytosis is more preferable than leucopenia that often achieves the critical values during radiation therapy and/or chemotherapy application.

A statistical reliable increase of the neutrophils number also has a disintoxication character ( $p < 0,01$ ) - from  $64,76 \pm 17,31\%$  at people of the basic group 1 to  $70,99 \pm 19,69\%$  at people of the basic group 2).

Last years the growth of infectious complications events at the oncological patients has the place in all countries because of the toughening of the specific treatment methods.

It wouldn't be exaggerated to say that the execution of chemotherapy itself takes relatively short period of time, the fight with infectious complications that have place after chemotherapy takes much more time.

The main causes of infectious complications development in oncological patients are reduction of phagocytes number (neutropenia) and phagocyte reaction disorders.

The most common infectious complications after chemotherapy are pneumonia, sepsis, and purulent infections.

Thus, the potentiating action on leucopoiesis (growth of leucocytes and neutrophils number in comparison with immunosuppressive influence of the specific therapy that increases the risk of an infectious complications appearance) should be considered as the doubtless advantage.

The increase in the relative basophils content that is observed at therapy by the "Normogen" can be explained by the reactions of allergization, which appear after introduction of exogenous immunoglobulins into the organism of the patient.

It is known that blood basophils play a part of target cells at the allergic reactions. They are a kind of histamine depot. At the same time the slight basophilia that is observed at the patients after the treatment with the "Normogen" preparation and that is an evidence of sensitization to proteins that are foreign to the humans, excludes a system anaphylaxis during which basophils also response by degranulation that can lead to the total basophils disappearance in the blood for a short time.

The parameter of the unspecific resistance is displayed in the table 2.

Table 2

The parameter of the unspecific resistance in oncology patients before and after the treatment with the “Normogen” preparation ( $X \pm m$ ).

Parameter	Basic group before the treatment (gr.1)	Basic group after the treatment (gr.2)	Control group (healthy, gr.3)
Hemolytic activity of the complement system	57,47±15,36	53,92±14,41	58,06±15,52
C3 –complement component	135,84±36,3	139,05±37,16	147,52±39,43
C4 - complement component	33,08±8,84	31,88±8,52	34,83±9,31
<b>HCT-test</b>			
NBT-test - spontaneous	11,16±2,98	8,68±2,32**	7,68±2,05
NBT-test – induced by zymoza	23,1±6,17	26,58±7,1*	37,54±10,03

Comment - \* -  $p < 0,05$ ; \*\* -  $p < 0,01$

The decrease of the hemolytical complement activity (that is observed at the people of the group 2 after the treatment with the “Normogen” preparation - 53,92±14,41% in comparison with 57,47±15,36% before the treatment) is explained by the “Normogen” preparation mechanism of action.

The “Normogen” preparation contains a set of polyclonal antibodies, which are antiidiotypical to alfa-protein and are capable to interact selectively with corresponding receptors of the tumor cell membranes. They also can bind and activate the complement components, inducing the lysis of the tumor cells.

As a result, the preparation efficiency remains only at normal values of hemolytical complement activity of all complement subunits (components). Since initial parameters of complement hemolytical activity and C3 and C4 of the complement components were within the range of normal parameters in the process of treatment with the “Normogen” preparation, it was often necessary to resort to the complement substitutive therapy (intravenous introduction of freshfrozen plasma and intact blood).

Immune complexes that form after introduction of the “Normogen” preparation (antigens of tumor cells + antiidiotypical antibodies to AFP) attract complement subunits tumor cells lysis execution. Thus, the complement activity decrease, which is observed at oncology patients after treatment with the “Normogen” preparation can be called as hipocomplementemia of the consumption.

The complement components C3 and C4 concentration changes is statistically doubtful. Unfortunately, detection of other complement subunits that is necessary for objective estimation of the complement system physiological activity is yet impossible.

NBT – test (nitro blue tetrazolium test) describe such important function of neutrophils as phagocytosis.

The phagocytosis system is considered as unspecific mechanisms, which limit the growth of tumor and not connected with specific antigens on the tumor cells.

Macrophages as well as T-lymphocytes, have critical importance in detection of specific tumor antigens.

Oncology patients before the treatment with the "Normogen" preparation have nitro blue tetrazolium increased reduction by neutrophils ( $11,16 \pm 2,98$ ) ( $p < 0,001$ ), that exceeds the analogous parameter at the people of the control group ( $7,68 \pm 2,05$ ) and indicate the increase in chemotaxis and devouring capacity of phagocytes.

These changes can be explained by activation of the unspecific defense mechanisms against tumor cells that leads to localization of the tumor.

Immunocorrection with the "Normogen" preparation resulted in statistically reliable decrease in nitro blue tetrazolium spontaneous reduction by neutrophils (spontaneous NBT test) that is an evidence of specific mechanisms engaging that destroy and eliminate tumor cells and that are triggered by influence of the preparation antitypical antibodies on AFP. This parameter in the basic group 2 (after the treatment) was close to the parameter of the control group ( $8,68 \pm 2,32$  and  $7,68 \pm 2,05$  respectively).

Nitro blue tetrazolium spontaneous reduction by neutrophils that were incubated with zymosan (induced NBT-test) was reliably higher in the oncology patients after treatment with the "Normogen" preparation ( $p < 0,05$ ) than before the treatment and this is an evidence of the phagocytosis reserve increase.

However, despite the increase, this parameter did not reach the parameter of the test in the people of the control group. At the same time, the tendency of NBT-test increase improves prognostic prospects and dictates the treatment continuation necessity.

The dynamics of changes in the cell immunity parameters is represented in the table 3

Table 3

**Cell immunity parameters in the oncology patients before and after the treatment with the "Normogen" preparation ( $\bar{X} \pm m$ )**

Cell immunity parameters:	Basic group before the treatment (gr.1)	Basic group before the treatment (gr.2)	Control group (healthy, gr.3)
T- lymphocytes (relat.)	$58,54 \pm 15,65$	$64,41 \pm 17,22^*$	$64,33 \pm 17,19$
T- lymphocytes (absol.)	$0,93 \pm 0,25$	$1,28 \pm 0,34^*$	$1,15 \pm 0,31$
T- helpers ( $T_H$ ) (relat.)	$36,74 \pm 9,82$	$40,68 \pm 10,85^*$	$37,22 \pm 9,95$
T- helpers ( $T_H$ ) (absol.)	$0,75 \pm 0,2$	$0,92 \pm 0,25^*$	$0,49 \pm 0,13$
T- suppressor ( $T_S$ ) (relat.)	$22,5 \pm 6,01$	$27,62 \pm 7,38^*$	$16,44 \pm 4,39$
T- suppressor ( $T_S$ ) (absol.)	$0,4 \pm 0,77$	$0,58 \pm 0,16^*$	$0,23 \pm 0,06$
$T_H/T_S$	$1,9 \pm 0,51$	$2,01 \pm 0,54^*$	$1,65 \pm 0,44$
B- lymphocyte (EAC) (relat.)	$9,4 \pm 2,51$	$11,52 \pm 3,08^*$	$15,9 \pm 4,25$
B- lymphocyte (EAC) (absol.)	$0,13 \pm 0,04$	$0,2 \pm 0,05^*$	$0,53 \pm 0,14$
0 lymphocyte (relat.)	$39,31 \pm 10,51$	$33,52 \pm 8,96^*$	$17,16 \pm 4,59$
0 lymphocyte (absol.)	$0,69 \pm 0,18$	$0,74 \pm 0,2$	$0,28 \pm 0,07$
Auto- lymphocyte (relat.)	$8,92 \pm 2,74$	$9,79 \pm 2,71^*$	$1,71 \pm 0,46$
Auto- lymphocyte (absol.)	$0,16 \pm 0,04$	$0,21 \pm 0,06^*$	$0,05 \pm 0,01$

Comment - \*  $p < 0,01$ ;

IRI – immuno-regulatory index.

It is known that parallel with macrophages and NK-cells, the leadership in detection of the specific tumor antigens belongs to T-lymphocytes that also have effector functions.

The "Normogen" preparation has a double effect on T- and B-lymphocytes of the patient: on the one hand it has a cytolytical effect directly on the tumor cells, on the other hand it executes the lysis of T-lymphocytes of the recipient with a helper activity for preservation of the tumor cells.

Our investigations revealed positive immunotropic action of the preparation on the parameters of cell immunity of the examined people in addition to direct cytolytical action on the tumor cells and pathological T- and B-lymphocytes.

Thus, the patients with oncopathology after immunocorrection with the "Normogen" preparation have a distinct increase in relative and absolute number of T-lymphocytes ( $p < 0,01$ ) from  $58,54 \pm 1,56\%$  and  $0,93 \pm 0,25$  in 1 liter to  $64,41 \pm 17,22\%$  and  $40,81 \pm 10,85$  in 1 liter respectively.

The leadership in induction and realization of the specific cytotoxic function to the tumor cells belongs to T-helpers, in particular to Tx1 and T-killers.

T-helpers recognize tumor antigens in association with MHC of 2 class on the surface of antigenpresented cells (macrophages and dendrite cells), then cytokines are activated and synthesized and later help to switch on a whole series of cell antitumor immunity mechanisms.

Thus the synthesized Tx1 IL-2 autocrinally stimulate the additional proliferation of T-helpers, reinforcing the intensity of the immune response.

In addition to that, IL-2 together with  $\gamma$ -interferon stimulates proliferation and differentiation of tumor specific T-killers.

In this connection, the productive immunostimulating effect with preemptive influence on T-helpers and T-suppressors can be considered as one of the main advantages of the "Normogen" preparation.

Thus, after the course of treatment with the "Normogen" preparation the relative and absolute number of T-helpers increased ( $p < 0,01$ ) from  $36,74 \pm 9,82\%$  and  $0,75 \pm 0,2$  in 1 liter to  $40,61 \pm 10,85\%$  and  $0,92 \pm 0,25$  in 1 liter respectively.

$\gamma$ -interferon, which is excreted by T-helpers and suppress growth of tumor cells, is, besides, the powerful endogenous immunomodulator and activate macrophages that results in increase of production of  $\alpha$ -interferon, TNF- $\alpha$ , IL-1 and other cytokines by them, that provide additional antitumor action.

The cell factors, which participate in antitumor immunity reactions, can possess not only positive function of malignant cell elimination, but also can reduce the defending efficiency from tumor antigens, inhibiting proliferation of T-killers. Activation of T-suppressors can be promoted by tumor antigen structure properties, and immunodepression that accompanies tumor growth and by other factors.

Tx2 (SD4+30+) perform suppressive function in antitumor immune response by secreting IL-2, IL-10, and IL-13 that inhibit activation of macrophages and NK-cells. T-suppressors (SD8+30+) act in analogous way, producing IL-4 and IL-10 that inhibit secretion of IL-2 and  $\gamma$ -interferon. As a result, proliferation of T-killers, which execute the malignant cells lysis, is decelerated.

The blockade of the antigen-recognizing receptors of T-helpers with participation of the antiidiotypical clones of T-suppressors is possible. Receptors of the antiidiotypical T-suppressors imitate space structure of tumor antigen molecules and block their functions interacting with receptors of T-helpers. The statistically significant ( $p < 0,01$ ) increase in the relative and absolute number of T-suppressors can be noted as one of the preparation effects. This fact can be considered as positive, because the maximum therapeutic influence of the "Normogen" preparation is observed at the parameter of the IRI (immunoregulatory index) 1.0 – 1.25, that represents the ratio of T-helpers and T-suppressors.

It is known, that adequately functioning immune system promptly destroy the majority

of appearing tumors, if they have not grown the critical number of  $10^8$ - $10^9$  tumor cell.

If at the initial stages of tumor development the immune system was suppressed because of any reason, or the tumor cells possessed a very high degree of tumor growth and overgrew these critical sizes, then at a later time the growth of the tumor surpasses the abilities of the immune system that leads to development of the clinically expressed forms of cancer.

According to tumor progression the immunodeficiency state of the patient get worse. In this connection the positive immunotropic effect of the "Normogen" preparation, which manifests in increase of T-lymphocytes and, first of all, T-helpers content, is worthy of notice.

The parameters of the humor immunity of the oncology patients are represented in the table 4.

Table 4

**The parameters of the humor immunity of the oncology patients before and after treatment with the "Normogen" preparation ( $\bar{X} \pm m$ ).**

<b>The parameters of the humor immunity:</b>	<b>Basic group before the treatment (gr.1)</b>	<b>Basic group before the treatment (gr.2)</b>	<b>Control group (healthy, gr.3)</b>
IgA (Immunoglobulin A)	328.93±87.91	338.09±90.36*	241.8±64.62
IgM (Immunoglobulin M)	147.68±39.47	179.05±47.85*	150.24±40.15
IgG (Immunoglobulin G)	1146.55±306.43	1165.26±311.43	1164.51±311.23
<b>Antitissue autoantibodies to the tissue:</b>			
Antitissue autoantibodies to the tissue: Heart	20,24±6,4	22,61±7,99	8,83±2,36
Antitissue autoantibodies to the tissue: Lung	22,65±6,83	30,66±10,84*	10,93±2,92
Antitissue autoantibodies to the tissue: Liver	10,43±3,69	11,65±4,76	10,65±2,85
Antitissue autoantibodies to the tissue: Intestines	19,39±6,13	25,7±8,13*	10,67±2,85
Circulating immune complexes	2,5±0,67	2,85±0,76	2±0,53

Comment - \*  $p < 0,05$

The humor immunity response against tumor antigens begins with synthesis of IgM. If IgM reaches the surface of the tumor cell and bind with accompanying antigens determinants, then in the later time the activation of the complement system occur by the classical way (one molecule IgM that binds an antigen is sufficient) and the lytical complexes, which forms during this process, destroy the tumor cell membrane.

The concentration of IgM in the observed patients - 147,68 +39,47mg% was, for certain, higher, than in the people of the control group - (150,24±40,15mg%). After immunocorrection with the "Normogen" preparation this parameter rise up to 179,05±47,85mg%. It is necessary to note, however, that IgM is effective, mainly, when the tumor substrate is in the circulatory system. If the tumor is out of the circulatory system, then IgM does not reach its surface (penta-dimensional molecule does not penetrate through the vascular wall) and because of this the lysis does not occur.

In the most of tumors the synthesis of IgM is rather quickly replaced by synthesis of IgG that rapidly permeates through the vascular wall and reaches the surfaces of the

tumor cells, but activation of the complement system and lysis of the tumor cell.

In our investigation the concentration of IgG during the process of treatment with the “Normogen” preparation did not undergo any significant changes. At the same time the statistically reliable increase in IgA concentration is registered that can inform about allergization of the organism.

Circulating immune complex concentration changes have inessential character. The reliable rise of level of antitissue autoantibodies to tissues of lung and intestine, most likely, is connected with localization of the tumor in these organs.

Circulating immune complexes can work as blocking factors. In this case antigen-recognizing T-lymphocytes are blocked, that reduces their antitumor activity.

Summarizing abovementioned, we can say that the part of the humor immunity in the antitumor defense is ambiguous. Most often, the antibodies synthesis does not have the decelerating influence on the neoplasms.

Thus, the analysis of the “Normogen” preparation efficiency showed the sufficiently high preparation efficiency in the correction of immunology shifts at oncology patients that manifests in immunity cell factors activation, phagocytosis potentiating effect and increase in IgM concentration. The changes in these parameters correlate both with state of health and general state of the patients. The advantage of the preparation is xenogeneic origin that provides the absence of parenteral infections contamination danger.

Immunograms graphic presentations of individual patients are represented in the annex 1.

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