Kul'chyns'kyi Andriy B, Zukow Walery. Three variants of immune responses to balneotherapy at the spa Truskavets' in patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018;8(3):476-489. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1209339
http://ojs.ukw.edu.pl/index.php/johs/article/view/5391

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eissn 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 05.02.2018. Revised: 10.02.2018. Accepted: 28.03.2018.

Three variants of immune responses to balneotherapy at the spa Truskavets' in patients with chronic pyelonephritis and cholecystitis

Andriy B Kul'chyns'kyi[1, 2], Walery Zukow[3]

[1]Communal Hospital, Truskavets', Ukraine akul@i.ua
[2]Ukrainian Scientific Research Institute of Transport Medicine Ministry of Health, Odesa, Ukraine prof.gozhenko@gmail.com
[3]Department of Spatial Management and Tourism, Faculty of Earth Sciences, Nicolaus Copernicus University, Torun, Poland w.zukow@wp.pl

Abstract

Background. The results of research the influence of balneotherapy in the spa Truskavets' on the immune status of the organism are ambiguous. This may be due to the differences between the cohorts of the subjects under investigation, and individual immune reactivity. Therefore, research in this direction remains relevant. The purpose of this study is to identify variants of the immunotropic effect of balneotherapy. **Material and methods.** In basal conditions in 33 men and 10 women with chronic pyelonephritis and cholecystitis in remission, we recorded twice, before and after balneotherapy at the spa Truskavets', parameters of Immune status on a set of I and II levels recommended by the WHO. **Results.** Three variants of immune responses to balneotherapy have been revealed. In 46,5% of patients changes in immune parameters fluctuated around the zero line and did not go beyond the limits of one Z-unit, that is, there was a quasi-zero immunotropic effect. In 32,6% of patients, moderate activation of neutrophil bactericidal activity was detected against as Staph. aur., and E. coli, an increase in the level of immunoglobulins G and M, as well as T-helpers, while the level of cytolytic T-lymphocytes was moderately reduced, in the absence of significant changes in the remaining 5 parameters of the immunogram. In 20,9% of patients, on the contrary, the level of T-killers has moderately increased, as well as the level of natural killers, in combination with a significant increase in neutrophil bactericidity against gram-positive and gram-negative bacteria, in the absence of significant changes in the remaining 7 parameters of the immunogram. **Conclusion.** Immune responses to balneotherapy have a polyvariant nature, which is probably due to individual reactivity, and will be the subject of the next study.

Keywords: Spa Truskavets', chronic pyelonephritis and cholecystitis, Immunity, Phagocytosis, Staphylococcus aureus, Escherichia coli.

INRODUCTION

The results of research the influence of balneotherapy in the spa Truskavets' (Ukraine) on the immune status both people and rats are ambiguous [3,6,7,9-15,23,25-29]. This may be due to the differences between the cohorts of the subjects under investigation (liquidators of the Chernobyl accident, residents of radionucleides polluted areas, individuals after radical treatment of oncopathology, women with hyperplasia of the thyroid gland as well as healthy rats), and individual immune reactivity. Therefore, research in this direction remains relevant. The purpose of this study is to identify variants of the immunotropic effect of balneotherapy.

MATERIAL AND METHODS

The object of observation were 33 men and 10 women aged 24-70 years old, who came to the spa Truskavets' for the treatment of chronic pyelonephritis combined with cholecystitis in remission. The survey was conducted twice, before and after ten-day balneotherapy (drinking of bioactive water of Naftussya, application of ozokerite, mineral baths).

In portion of capillary blood we counted up Leukocytogram and calculated its Adaptation Index as well as Strain Index by IL Popovych [2,20,21].

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manual [18]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD16 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Immunoglobulins classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method).

We calculated also the Entropy (h) of Immunocytogram (ICG) and Leukocytogram (LCG) using classical CE Shannon's formula [24]:

$$hICG= - [CD4\bullet\log_2 CD4 + CD8\bullet\log_2 CD8 + CD22\bullet\log_2 CD22 + CD16\bullet\log_2 CD16]/\log_2 4$$

$$hLCG= - [L\bullet\log_2 L + M\bullet\log_2 M + E\bullet\log_2 E + SNN\bullet\log_2 SNN + StubN\bullet\log_2 StubN]/\log_2 5$$

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [5] with moderately modification by MM Kovbasnyuk [17]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". Take into account the following parameters of Phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index PhI), intensity (number of microbes absorbed one phagocytes - Microbial Count MC or Right's Index) and completeness (percentage of dead microbes - Killing Index KI). On the basis of the recorded partial parameters of Phagocytosis, taking into account the Neutrophils (N) content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula [22,27]:

$$BCCN\ (10^9\ Bact/L) = N\ (10^9/L)\bullet PhI\ (\%)\bullet MC\ (Bact/Phag)\bullet KI\ (\%)\bullet 10^{-4}$$

Norms are borrowed from the database of the Truskavets' Scientific School of Balneology.

Results processed by methods of cluster and discriminant analyses, using the software package "Statistica 5.5".

The results are presented at the conference [16].

RESULTS AND DISCUSSION

The cornerstone of the assessment of the effectiveness of balneotherapy is the study of changes in the immune system as being responsible for the termination of chronic inflammatory process in the kidneys and bile duct, supported by the conditionally pathogenic microflora, primarily Escherichia coli and Staphylococcus aureus. Preliminary analysis has shown that in different patients, individual immunity parameters respond to balneotherapy not only in varying degrees, but even in the opposite way. In order to evaluate the immune responses on a single scale according recommendation by IL Popovych [22,23,27] immune variables (V) expressed as Z-scores calculated by formula:

Z=(V/N – 1)/Cv, where

N is Mean of Normal Variable,

Cv is Coefficient its variation.

The second phase was conducted Cluster analysis of Immune responses expressed as Z-scores. Use of Cluster analysis makes possible the simultaneous consideration of all the signs. Considering the totality of characteristics of persons undertaken in their relationship and conditionality of some of these (derivatives) other (main determinants) allows as to make a natural classification that reflects the nature of things, their essence. It is believed that knowledge of the essence of the object is to identify those of its quality properties that actually define the object, distinguish it from other [1,19].

Clustering cohort of persons is realized by iterative k-means metod. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points [1,19].

Distribution of patients between clusters is presented in Table 1.

Table 1. Members of Cluster and Distances from Respective Cluster Center

Cluster Number 3, contains 9 cases

	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
	C_4	C_7	C_13	C_14	C_15	C_27	C_28	C_31	C_37
Distance	1,4	0,8	1,7	1,1	2,3	1,4	1,6	1,0	1,3

Cluster Number 2, contains 14 cases

	Case No	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
	C_1	C_2	C_3	C_5	C_6	C_8	C_9	C_10	C_11	C_16	C_23	C_25	C_30	C_40
Distance	1,1	1,4	1,0	1,0	0,9	1,2	1,5	1,6	1,4	1,6	1,4	1,7	1,4	2,1

Cluster Number 1, contains 20 cases

	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
	12	17	18	19	20	21	22	24	26	29	32	33	34	35	36
Distance	1,5	1,2	0,9	0,6	1,1	1,0	0,9	1,6	2,0	1,2	1,0	1,8	1,2	0,8	0,9

	Case No.	Case No.	Case No.	Case No.	Case No.
	38	39	41	42	43
Distance	1,3	0,8	1,9	1,5	2,3

At the next stage of the analysis, immune profiles of each cluster were created (Fig. 1).

As we see, in 46,5% of patients (green line) changes in immune parameters fluctuated around the zero line and did not go beyond the limits of one Z-unit, that is, there was a quasi-zero immunotropic effect.

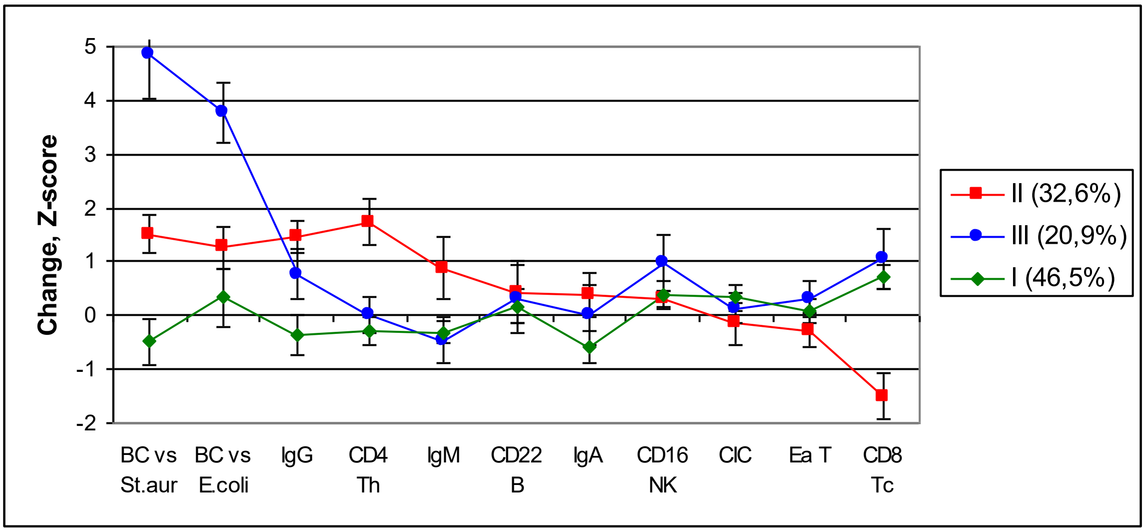


Fig. 1. Three variants of immune responses to balneotherapy at the resort Truskavets

In 32,6% of patients (red line), moderate activation of neutrophil bactericidal activity was detected against as Staph. aur., and E. coli, an increase in the level of immunoglobulins G and M, as well as T-helpers, while the level of cytolytic T-lymphocytes was moderately reduced, in the absence of significant changes in the remaining 5 parameters of the immunogram. In 20,9% of patients (blue line), on the contrary, the level of cytolytic T-lymphocytes (T-killers) has moderately increased, as well as the level of natural killers, in combination with a significant increase in neutrophil bactericidity against gram-positive and gram-negative bacteria, in the absence of significant changes in the remaining 7 parameters of the immunogram.

In order to evaluate immunity in general, we calculated the integral immune index as the average for 11 immune Z-scores taking into account their signs. The difference between the endpoints and the initial values was considered a measure of the integral immunotropic effect. As we see in Fig. 2, in patients of the first cluster, the immune status was completely normal (therefore indicated by green) even at admission, which is not surprising given the phase of remission of the disease. The immune status has not changed and after the course of balneotherapy, which is consistent with the long-established position that the natural therapeutic factors exhibit their normalizing effects, in particular immunotropic, only against the background of deviations from the norm. Instead, in patients of two other clusters, a moderate immunodeficiency was noted, indicated by a yellow color, which, under the influence of the balneotherapy course, was reduced to the normal zone (plus or minus one Z-unit). In this case, the integral immunotropic effect in the third cluster was more pronounced than in the second.

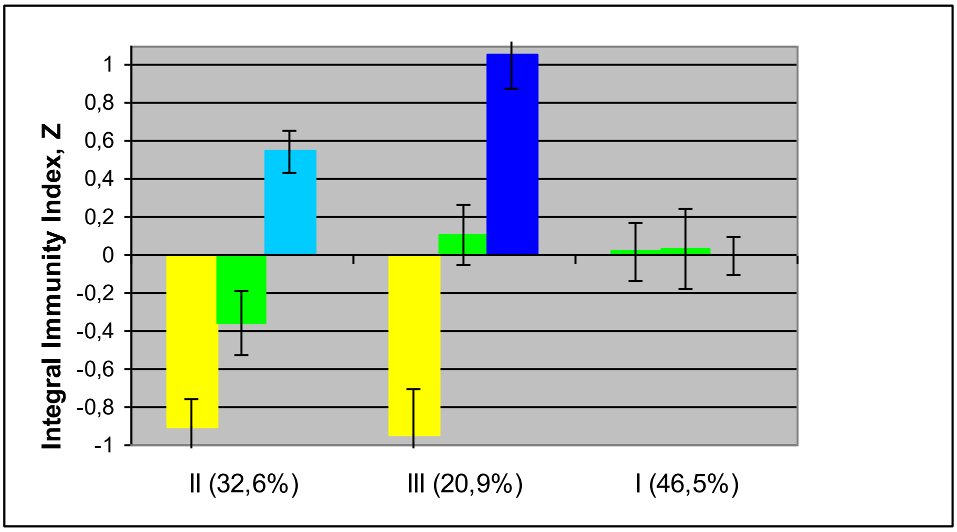
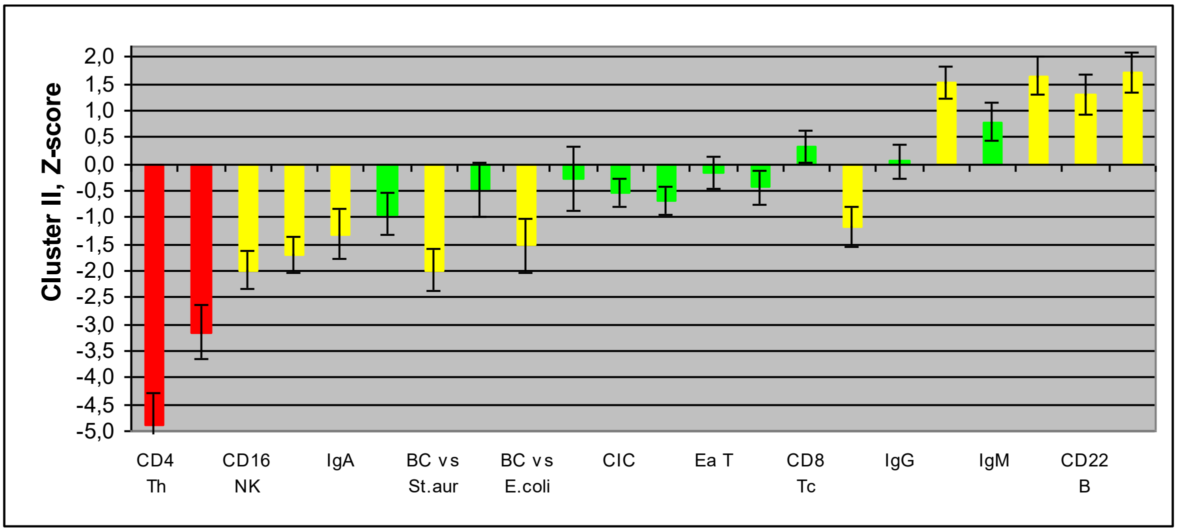


Fig. 2. Integral state of immunity before (first column) and after (second column) balneotherapy and its changes (third column) in the patients of three clusters

Figure 3 shows the so-called immune profiles, that is, the state of individual parameters of the immunogram to (first columns) and after (second columns) of balneotherapy. As we see, the characteristic feature of the immunotropic effect in the second cluster is a reduction in the deficiency of T-helper cells and the complete normalization of bactericidity of the neutrophils. The third cluster is characterized by the total normalization of levels of T-killers and natural killers and displacement of levels of bactericidity from the deficiency zone to the upper zone of the norm. In the members of the first cluster, most of the parameters remain stable normal, and the level of T-helpers remain moderately reduced.



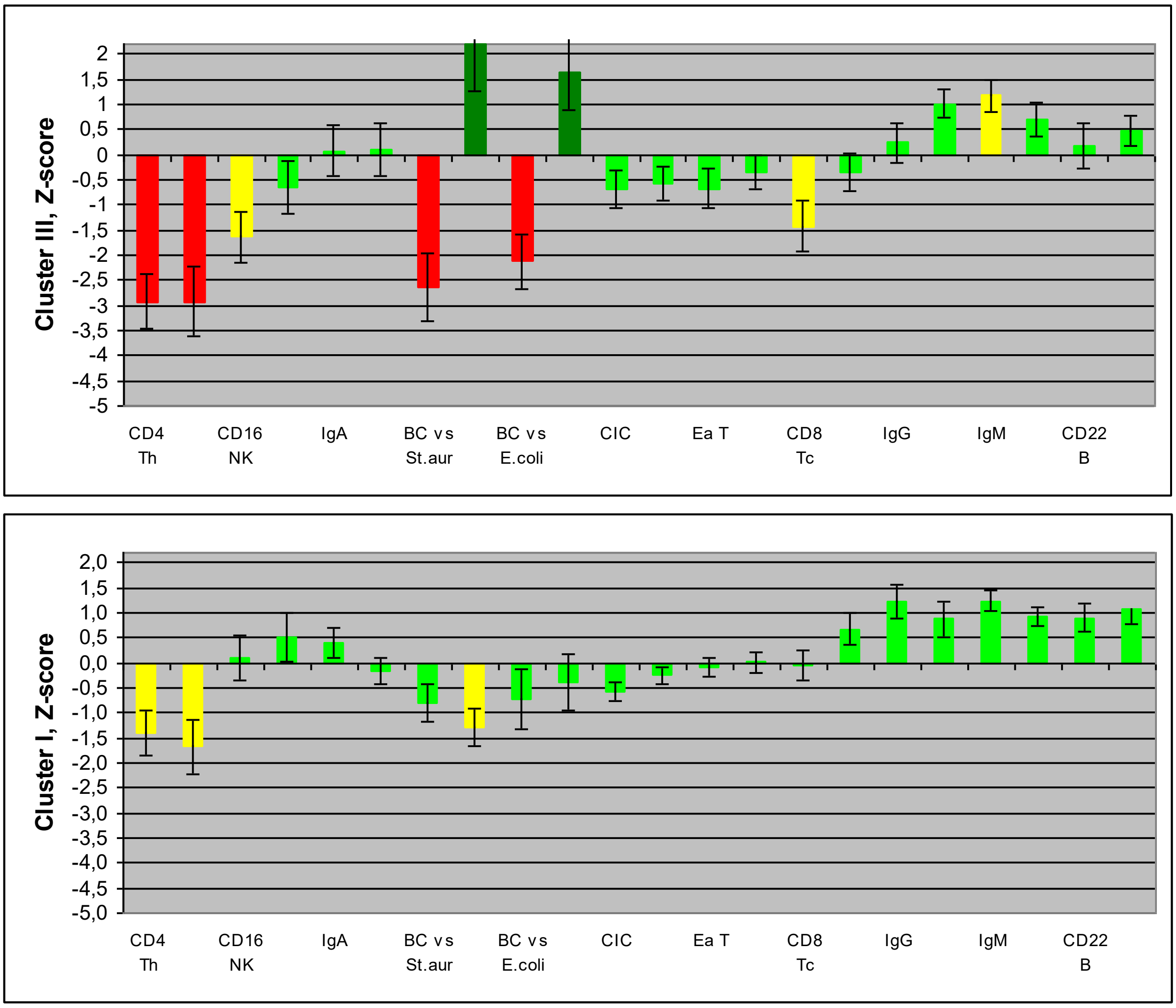


Fig. 3. Immune profiles before (first columns) and after (second columns) of balneotherapy

In order to identify individual immune responses, a method of discriminant analysis (forward stepwise) was used [8]. The program has selected (included in the model) 7 so-called discriminant (recognizing, separating) immune parameters (Tables 2-5), the information on which changes are further consolidated into two canonical (generalizing) roots. In this case, the first root contains 70% of recognition capabilities and reflects the inverse nature changes in bactericidity with respect to Staph. aureus and the integral immune index, while the second root also inversely reflects changes in blood levels of T-killers, natural killers and high-affinity ("active") T-lymphocytes, instead of a direct change in the level of T-helper cells. As you can see, clusters are separated, but not quite clearly.

Table 2. Discriminant Function Analysis Summary
Step 7, N of vars in model: 7; Grouping: 3 grps
Wilks' Lambda: 0,1366; approx. $F_{(15)}$=8,3; p<10^{-6}

Variables, changes in which are expressed as Z-scores, currently in the model	Wilks' Λ	Partial Λ	F-remove $_{(2,34)}$	p-level	Tolerancy
Bacterocidity vs Staphylococus aureus	,165	,826	3,6	,039	,563
$CD8^+$ T cytolytic Lymphocytes	,173	,788	4,6	,017	,591
Integral Immunity Index-11	,188	,727	6,4	,004	,311
Circulating Immune Complexes	,157	,867	2,6	,089	,795
"Active" T Lymphocytes	,146	,933	1,2	,305	,662
$CD16^+$ NK Lymphocytes	,153	,892	2,1	,143	,621
$CD4^+$ T helper Lymphocytes	,149	,916	1,6	,224	,627

Variables currently not in the model (Df for all F-tests: 2,33)

	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Bacterocidity vs Escherichia coli	,134	,980	,335	,717	,701
$CD22^+$ B Lymphocytes	,135	,988	,204	,817	,897
IgG	,135	,991	,152	,860	,693
IgA	,131	,959	,699	,504	,714
IgM	,133	,972	,473	,627	,599

Table 3. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Lambda	F-value	p-level
Bacterocidity vs Staphylococcus aureus	25,2	10^{-6}	,442	25,2	10^{-6}
$CD8^+$ T cytolytic Lymphocytes	13,8	10^{--4}	,259	18,8	10^{-6}
Integral Immunity Index-11	7,0	,003	,189	16,5	10^{-6}
Circulating Immune Complexes	2,1	,136	,170	13,2	10^{-6}
"Active" T Lymphocytes	1,2	,299	,159	10,9	10^{-6}
$CD16^+$ NK Lymphocytes	1,1	,329	,149	9,3	10^{-6}
$CD4^+$ T helper Lymphocytes	1,6	,224	,137	8,3	10^{-6}

Table 4. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	**Standardized**		**Raw**	
Variables currently in the model	Root 1	Root 2	Root 1	Root 2
Bacterocidity vs Staphyloc. aureus	-,197	-,733	-,101	-,375
Integral Immunity Index-11	-1,090	,250	-2,431	,558
Circulating Immune Complexs	,458	-,183	,383	-,153
$CD8^+$ T cytolytic Lymphocytes	,622	-,398	,452	-,289
$CD16^+$ NK Lymphocytes	,115	-,560	,104	-,508
"Active" T Lymphocytes	,374	-,060	,345	-,055
$CD4^+$ T helper Lymphocytes	-,115	,489	-,090	,380
	Constants		1,033	,391
Chi-Square Tests with Successive Roots Removed				
Eigenvalues			2,49	1,10
Canonical R			,845	,723
Wilks' Lambda			,137	,477
Chi-Square			74	27
Degree freedom			14	6
p-level			10^{-6}	10^{-4}

Table 5. Changes in Discriminant Variables and correlations Variables - Canonical Roots

Changes in Variables, Z	Cluster I	Cluster II	Cluster III	Root 1	Root 2
Bacterocidity vs Staph. aureus	**-0,49±0,42**	+1,51±0,36	+4,85±0,83	**-,574**	-,634
Integral Immunity Index-11	**0,00±0,10**	+0,55±0,11	+1,06±0,18	**-,557**	-,432
Circulating Immune Complexs	**0,33±0,24**	-0,15±0,40	0,13±0,28	**,103**	-,083
$CD8^+$ Tc Lymphocytes	+0,72±0,23	**-1,50±0,42**	**+1,06±0,57**	,294	**-,654**
$CD16^+$ NK Lymphocytes	+0,40±0,24	**+0,29±0,18**	**+0,98±0,53**	-,045	**-,222**
"Active" T Lymphocytes	+0,09±0,22	**-0,27±0,33**	**+0,31±0,35**	,041	**-,189**
$CD4^+$ Th Lymphocytes	-0,28±0,26	**+1,74±0,42**	**0,00±0,34**	-,344	**,475**
			Discriminant Properties, %	**69,5**	**30,5**

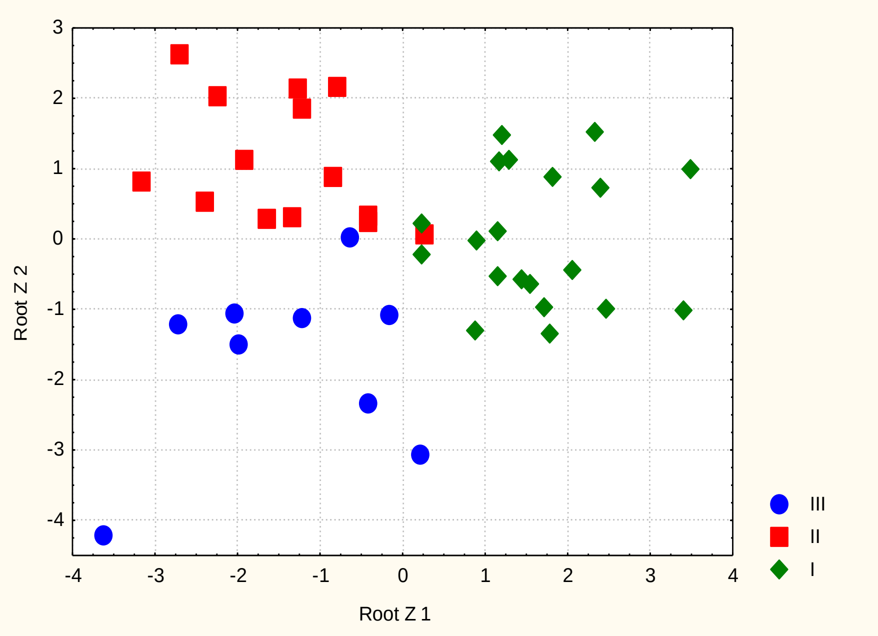


Fig. 4. Individual scores of discriminant roots of immune responses to balneotherapy in patients of different clusters

At the final stage to the discriminant analysis we also attracted the components of the leukocyte formula, which also relate to immunity. In this case, unlike the previous one, changes in immune parameters were expressed in actual units. In the discriminatory model included, with 11 parameters of the immunogram, also Shannon's Entropy of immunocytes, the total content in the blood of Leukocytes, the content of Monocytes in leukocytogram, as well as leukocytary Popovych's Strain and Adaptation indices (Tables 6 and 7).

The calculation of individual scores of discriminant roots of responses by summing the products of individual variables into Raw coefficients and Constants (Table 8) makes it possible to visualize all immune responses on the plane of the two roots (Fig. 5).

The localization of representative points of members of the third cluster along the first root axis in its extreme left region reflects the maximum for an increase in cohorts of those immune parameters that correlate with the root negatively. The shift of the second cluster to the right reflects less pronounced favorable changes in immune parameters or the absence of significant changes, while the extreme right of localization of the members of the first cluster reflects a lack of change or an unfavorable tendency.

On the other hand, along the axis of the second root, the second cluster occupies an extreme upper position, while the other two are placed at approximately the same level. Such a disposition reflects, first of all, a significant decrease in the level of T-killers in members of the second cluster, while in other clusters their level increases. The other 7 parameters, which correlate with the second root positively, increase or tend to increase in members of the second cluster, whereas in the other two clusters, they generally do not significantly change (Table 9).

Table 6. Discriminant Function Analysis Summary

Step 16, N of vars in model: 16; Grouping: 3 grps

Wilks' Lambda: 0,0274; approx. $F_{(34)}$=7,12; $p<10^{-6}$

Variables currently in the model	Wilks' Lambda	Partial Lambda	F-remove $_{(2,24)}$	p-level
Bactericidity vs Staph. aureus, 10^9 Bac/L	,030	,923	1,0	,381
$CD8^+$ T cytolytic Lymphocytes, %	,051	,536	10,4	,001
Popovych's Strain Index-2	,029	,929	0,9	,415
Shannon's Entropy of Immunocytogramm	,044	,619	7,4	,003
$CD22^+$ B Lymphocytes, %	,032	,846	2,2	,135
Killing Index vs Staph. aureus, %	,053	,517	11,2	$<10^{-3}$
IgA, g/L	,037	,748	4,0	,031
Bactericidity vs Escher. coli, 10^9 Bacter/L	,044	,628	7,1	,004
IgM, g/L	,040	,688	5,4	,011
Phagocytosis Index vs Staph. aureus, %	,038	,714	4,8	,018
Microbian Count for St. aur., Bacteras/Phag	,041	,659	6,2	,007
Microbian Count for E. coli, Bac/Phagocyte	,036	,752	4,0	,033
Monocytes, %	,033	,830	2,5	,107
Popovych's Adaptation Index-2, points	,033	,831	2,4	,109
Leukocytes, 10^9/L	,033	,825	2,5	,099
IgG, g/L	,030	,915	1,1	,346

Table 7. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Lambda	F-value	p-level
Bactericidity vs Staph. aureus, 10^9 Bac/L	25,2	10^{-6}	,442	25,2	10^{-6}
$CD8^+$ T cytolytic Lymphocytes, %	13,8	10^{-4}	,259	18,8	10^{-6}
Popovych's Strain Index-2	5,6	,008	,200	15,6	10^{-6}
Shannon's Entropy of Immunocytogramm	4,2	,023	,164	13,6	10^{-6}
$CD22^+$ B Lymphocytes, %	3,5	,043	,137	12,2	10^{-6}
Killing Index vs Staph. aureus, %	4,6	,016	,109	11,9	10^{-6}
IgA, g/L	3,1	,057	,092	11,2	10^{-6}
Bactericidity vs Escher. coli, 10^9 Bac/L	3,2	,054	,077	10,8	10^{-6}
IgM, g/L	2,0	,157	,068	10,0	10^{-6}
Phagocytosis Index vs Staph. aureus, %	2,5	,095	,059	9,7	10^{-6}
Microbian Count for Staph. aur., Bacter/Phag	1,2	,324	,055	8,9	10^{-6}
Microbian Count for E. coli, Bac/Phagocyte	1,1	,356	,046	7,9	10^{-6}
Monocytes, %	2,9	,075	,038	8,0	10^{-6}
Popovych's Adaptation Index-2, points	1,1	,347	,035	7,6	10^{-6}
Leukocytes, 10^9/L	2,0	,155	,030	7,5	10^{-6}
IgG, g/L	1,1	,346	,027	7,1	10^{-6}

Table 8. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized		Raw	
Variables currently in the model	**Root 1**	**Root 2**	**Root 1**	**Root 2**
Bactericidity vs Staph. aureus, 10^9 Bac/L	,767	-,246	,039	-,012
$CD8^+$ T cytolytic Lymphocytes, %	,982	-,759	,220	-,170
Popovych's Strain Index-2	-,852	-,659	-2,094	-1,619
Shannon's Entropy of Immunocytogramm	-1,352	,215	-32,808	5,212
$CD22^+$ B Lymphocytes, %	,718	,281	,115	,045
Killing Index vs Staph. aureus, %	-2,541	-,104	-,347	-,014
IgA, g/L	-,729	,195	-1,586	,423
Bactericidity vs Escherichia coli, 10^9 Bac/L	-1,192	-,156	-,061	-,008
IgM, g/L	,739	,811	1,787	1,962
Phagocytosis Index vs Staph. aureus, %	,611	,613	,600	,602
Microbian Count for Staph. aur., Bac/Phag	-1,676	,014	-,178	,002
Microbian Count for E. coli, Bac/Phagocyte	-1,033	-,529	-,088	-,045
Monocytes, %	-,362	-1,138	-,088	-,275
Popovych's Adaptation Index-2	-,613	,112	-1,147	,210
Leukocytes, 10^9/L	-1,089	,472	-,982	,425
IgG, g/L	-,218	,418	-,056	,108
		Constants	**3,083**	**,049**
Chi-Square Tests with Successive Roots Removed				
Eigenvalues			9,89	2,36
Canonical R			,953	,838
Wilks' Lambda			,027	,298
Chi-Square			115	39
Degree freedom			34	16
p-level			10^{-6}	0,001

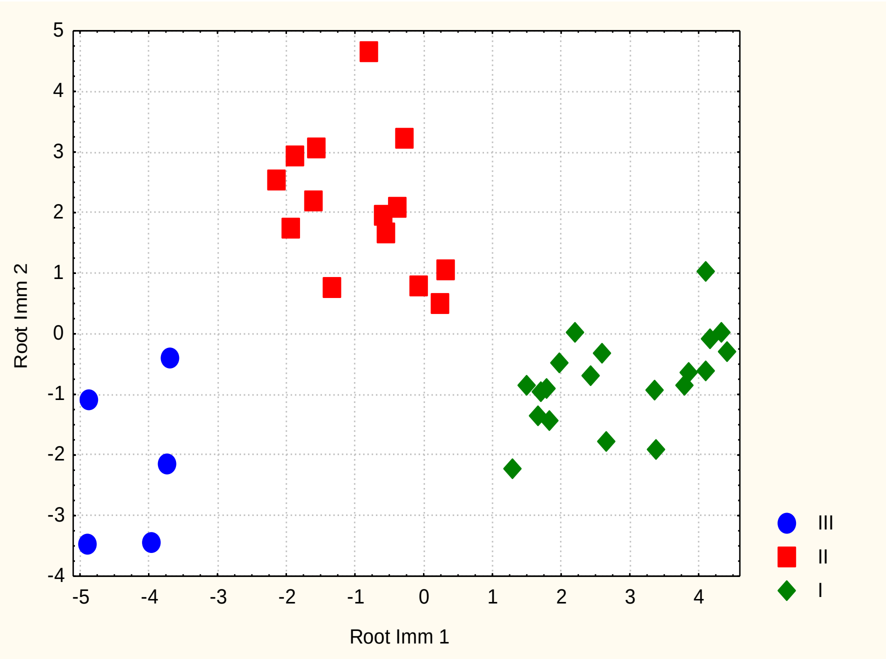


Fig. 5. Individual scores of discriminant roots of responses to balneotherapy parameters of immunity in patients of different clusters

Table 9. Changes in Immune Variables and correlations Variables - Canonical Roots (Pooled-within-groups correlations)

Change in Variables currently in the model	Cluster III (20,9%)	Cluster II (32,6%)	Cluster I (46,5%)	Root 1	Root 2
Bactericidity vs Staph. aureus, 10^9 Bac/L Z	+51,2±8,8 +4,85±0,83	+16,0±3,9 +1,51±0,36	-5,2±4,4 -0,49±0,42	**-,354**	-,093
Killing Index vs Staph. aureus, % Z	+14,0±1,6 +3,34±0,38	+7,7±2,3 +1,84±0,55	+0,9±1,6 +0,22±0,39	**-,231**	,022
Bactericidity vs Escherichia coli, 10^9 Bact/L Z	+37,3±5,5 +3,77±0,56	+12,5±3,8 +1,26±0,38	+3,2±5,2 +0,33±0,53	**-,213**	-,113
Leukocytes, 10^9/L Z	+0,83±0,44 +1,65±0,89	+0,04±0,24 +0,09±0,47	-0,51±0,26 -1,01±0,51	**-,151**	-,025
Shannon's Entropy of Immunocytogramm Z	+0,04±0,02 +0,75±0,34	+0,01±0,01 +0,16±0,20	+0,01±0,01 +0,18±0,12	**-,087**	-,127
Monocytes, % Z	+0,9±1,2 +1,78±2,35	-0,4±1,4 -0,70±2,75	-1,2±0,8 -2,35±1,61	**-,062**	-,013
Microbian Count for Staph. aur., Bac/Phag Z	+2,4±3,4 +0,49±0,68	+0,8±2,0 +0,17±0,41	-0,6±2,3 -0,13±0,46	**-,041**	,000
Popovych's Leukocytary Adaptation Index Z	+0,29±0,12 +1,18±0,48	+0,22±0,21 +0,89±0,83	+0,13±0,08 +0,53±0,32	**-,039**	,008
$CD8^+$ T cytolytic Lymphocytes, % Z	+3,4±1,8 +1,06±0,57	**-4,9±1,4** **-1,50±0,42**	+2,4±0,7 +0,72±0,23	,024	**-,537**
IgG, g/L Z	+2,0±1,2 +0,77±0,47	**+3,8±0,8** **+1,46±0,29**	-0,9±1,0 -0,36±0,38	-,124	**,267**
IgM, g/L Z	-0,13±0,12 -0,47±0,43	**+0,24±0,16** **+0,87±0,58**	-0,09±0,05 -0,31±0,20	-,013	**,259**
Phagocytosis Index vs Staph. aureus, % Z	-0,11±0,26 -0,13±0,29	**+0,65±0,23** **+0,74±0,26**	+0,19±0,27 +0,22±0,30	,019	**,185**
Popovych's Leukocytary Strain Index-2	-0,28±0,24	**+0,03±0,05**	-0,17±0,07	,014	**,188**
IgA, g/L Z	0,00±0,17 0,00±0,56	**+0,12±0,13** **+0,38±0,40**	-0,18±0,09 -0,57±0,30	-,065	**,142**
Microbian Count for Escher. coli, Bac/Phag Z	-3,4±3,2 -0,63±0,60	**+0,2±2,4** **+0,03±0,45**	-0,7±3,2 -0,13±0,60	,023	**,056**
$CD22^+$ B Lymphocytes, % Z	+1,1±2,2 +0,32±0,63	**+1,5±2,0** **+0,43±0,57**	+0,6±1,1 +0,17±0,32	-,014	**,032**

Note. For each variable, changes are made both in actual units and in the form of Z-units.

The clear separation of clusters in the information space of two roots is documented by calculation of Squared Mahalanobis Distances between Clusters (Table 10).

Table 10. Squared Mahalanobis Distances between Clusters and F-values (df=17,2). For all $p<10^{-4}÷10^{-6}$

Cluster	III	II	I
III	0	32	66
II	**5,6**	0	24
I	**13,2**	**6,5**	0

Selected 16 parameters can be used to identify (classify) the belongings of a particular patient to one or another immune response cluster. This purpose of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 11).

These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. The coefficients of the classifying functions are not standardized, therefore they are not interpreted. An object belongs to a group with the maximum value of a function calculated by summing the values of the variables by the coefficients of the classifying functions plus the constant. In this case, we can retrospectively recognize the belonging to each cluster unmistakably

Table 11. Coefficients and Constants for Classification Functions

Variables currently in the model	Clu III (20,9%)	Clu II (32,6%)	Clu I (46,5%)
Bactericidity vs Staph. aureus, 10^9 Bac/L	-,354	-,242	-,062
$CD8^+$ T cytolytic Lymphocytes, %	-1,434	-1,160	,149
Popovych's Strain Index-2	20,97	6,573	3,316
Shannon's Entropy of Immunocytogramm	252,9	138,7	,704
$CD22^+$ B Lymphocytes, %	-,923	-,292	,012
Killing Index vs Staph. aureus, %	2,970	1,509	,245
IgA, g/L	11,80	6,906	-,254
Bactericidity vs Escher. coli, 10^9 Bac/L	,538	,260	,052
IgM, g/L	-17,75	-3,347	-2,216
Phagocytosis Index vs Staph. aureus, %	-5,297	-,668	-,127
Microbian Count for Staph. aur., Bac/Phag	1,507	,791	,119
Microbian Count for E. coli, Bac/Phag	,798	,279	,078
Monocytes, %	1,112	-,247	,207
Popovych's Adaptation Index-2, points	10,06	6,171	1,268
Leukocytes, 10^9/L	7,492	5,056	,160
IgG, g/L	,292	,456	-,060
Constants	-35,68	-11,63	-1,629

The next article will demonstrate that each of the three types of immune response to balneotherapy is due to constellation of the initial parameters of the neuroendocrine-immune complex and metabolism and is predicted unmistakably.

To conclude this article we consider it necessary to state that the discovery of the variety of immunotropic effects of balneotherapy is a separate manifestation of the concept of the diversity of the effects of balneotherapy on the body as a whole [4,15,22,23].

ACKNOWLEDGMENT

We express our sincere gratitude to administration JSC "Truskavets'kurort" for help in carrying out immune tests.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

For all authors any conflict of interests is absent.

References

1. Aldenderfer MS, Blashfield RK. Cluster analysis (Second printing, 1985) [trans. from English in Russian]. In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika. 1989: 139-214.
2. Barylyak LG., Malyuchkova RV, Tolstanov OB, Tymochko OB, Hryvnak RF, Uhryn MR. Comparative estimation of informativeness of leucocytary index of adaptation by Garkavi and by Popovych. Medical Hydrology and Rehabilitation. 2013; 11(1): 5-20.

3. Bul'ba AY. Immune accompaniment of thyrotropic effects of balneotherapy in the spa Truskavets' in women with hyperplasia of the thyroid gland [in Ukrainian]. Medical Hydrology and Rehabilitation. 2008; 6(3): 60-65.
4. Chebanenko OI, Chebanenko LO, Popovych IL. Multivariate Balneoeffects of Factors of Spa Truskavets' and Forecasting [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2012: 496 p.
5. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil. 1981. 110 p.
6. Fuchko OL. Concomitant changes in immune status in women with chronic cholecystitis, by different thyrotropic effects of bioactive water Naftussya and possibility of their prediction [in Ukrainian]. Medical Hydrology and Rehabilitation. 2010; 8(3): 69-78.
7. Khodak OL, Bilas VR, Nazarenko NK. Variants of immunotropic and clinical effects of balneotherapy in the spa Truskavets' in individuals after radical treatment of oncopathology [in Ukrainian]. Medical Hydrology and Rehabilitation. 2006; 4(3): 9-32.
8. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskwa: Finansy i Statistika. 1989: 78-138.
9. Kozyavkina NV. Імунний аккомпанемент поліваріантних тиротропних ефектів біоактивної води Нафтуся у щурів. Immune accompaniment of polyvariant thyrotropic effects of bioactive water Naftussya in rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(2): 27-39.
10. Kozyavkina NV. Polyvariance of thyrotropic effects of bioactive water Naftussya in women with chronic endocrine-gynecological pathology, their neuroendocrine-immune and clinical support and prediction capabilities [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 4-23.
11. Kozyavkina NV. Thyrotropic effects of bioactive water Naftussya in female rats and their metabolic, neuroendocrine and immune accompaniments [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(4): 91-113.
12. Kozyavkina OV. Vegetotropic effects of bioactive water Naftussya in children with dysfunction of the neuroendocrine-immune complex, their endocrine-immune accompaniment and the possibility of prediction [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 23-38.
13. Kozyavkina OV. Vegetotropic effects of bioactive water Naftussya in male rats and their endocrine, electrolyte and immune accompaniments [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(3): 65-92.
14. Kozyavkina OV, Popovych IL, Zukow W. Immediate vegetotropic effects of bioactive water Naftussya and those neuro-endocrine-immune accompaniment in healthy men. Journal of Health Sciences. 2013; 3(5): 391-408.
15. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG, Popovych IL. Bioactive Water Naftussya and Neuroendocrine-Immune Complex [in Ukrainian]. Kyiv: UNESCO-SOCIO; 2015: 349 p.
16. Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Kovbasnyuk MM, Korolyshyn TA, Popovych IL. Immune responses to balneotherapy at the spa Truskavets' at patients with chronic pyelonephritis and cholecystitis, their neuro-endocrine mechanism and associated changes in metabolism, intestinal and urine microbiocenosis and cholekinetics. In: Proceedings X Scientific Conference "Issues of pathology in conditions of extreme factors action on the body" (Ternopil', 5-6 October 2017). Ternopil', 2017: 57-58.
17. Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Kyjenko VM, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 2. Correlations between parameters EEG, HRV and Phagocytosis. Journal of Education, Health and Sport. 2016; 6(10): 377-401.
18. Lapovets' LYe, Lutsyk BD. Handbook of Laboratory Immunology [in Ukrainian]. L'viv. 2002. 173 p.
19. Mandel ID. Cluster analysis [trans. from English in Russian]. Moskva: Finansy i Statistika. 1988. 176 p.
20. Petsyukh SV, Petsyukh MS, Kovbasnyuk MM, Barylyak LG, Zukow W. Relationships between Popovych's Adaptation Index and parameters of ongoiging HRV and EEG in patients with

chronic pyelonephrite and cholecystite in remission. Journal of Education, Health and Sport. 2016; 6(2): 99-110.
21. Popovych IL (editor). General Adaptation Reactions and Body's Resistance in Liquidators of the Chernobyl Accident [in Ukrainian]. Kyiv: Computerpress. 2000. 117 p.
22. Popovych IL. Stresslimiting Adaptogene Mechanism of Biological and Curative Activity of Water Naftussya [in Ukrainian]. Kyiv: Computerpress. 2011. 300 p.
23. Popovych IL, Vis’tak (Markevych) HI, Humega MD, Ruzhylo SV. Vegetotropic Effects of Bioactive Water Naftussya and their Neuroendocrine-Immune, Metabolic and Hemodynamic Accompaniments [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2014: 162 p.
24. Shannon CE. Works on the theory of informatics and cybernetics [transl. from English to Russian]. Moskwa: Inostrannaya literatura; 1963: 329 p.
25. Struk ZD. Multivariable immunotropic effects of bioactive water of Naftussya under drinking monotherapy conditions [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(2): 92-96.
26. Struk ZD. Neuroendocrine and clinical accompaniments of multivariate immunotropic effects of bioactive water Naftussya [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(4): 51-65.
27. Sydoruk NO, Chebanenko OI, Popovych IL, Zukow W. Comparative Investigation of Physiological Activity of Water Naftussya from Truskavets’ and Pomyarky Deposits [in Ukrainian]. Kyiv: UNESCO-SOCIO; 2017: 216 p.
28. Vis’tak HI. Endocrine and immune accompaniments of polyavariant vegetotonic effects of bioactive water Naftussya in women [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 81-85.
29. Vis’tak HI, Markevych RO. Polyvariance of the effects of bioactive water Naftussya on the vegetative reactivity, their endocrine and immune accompaniments and the possibility of prediction [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(4): 43-50.
30.