Gozhenko Anatoliy I, Hrytsak Yaroslav L, Barylyak Liliya G, Kovbasnyuk Marta M, Tkachuk Svitlana P, Korolyshyn Tetyana A, Matiyishyn Galyna Yo, Zukow Walery, Popovych Igor L. Features of immunity by various constellations of principal adaptation hormones and autonomous regulation in practically healthy people. Journal of Education, Health and Sport. 2016;6(10):215-235. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.160979
http://ojs.ukw.edu.pl/index.php/johs/article/view/3936

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 755 (23.12.2015).
755 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: 02.10.2016. Revised 02.10.2016. Accepted: 15.10.2016.

FEATURES OF IMMUNITY BY VARIOUS CONSTELLATIONS OF PRINCIPAL ADAPTATION HORMONES AND AUTONOMOUS REGULATION IN PRACTICALLY HEALTHY PEOPLE

Anatoliy I Gozhenko[1], Yaroslav L Hrytsak[2], Liliya G Barylyak[2,3,4], Marta M Kovbasnyuk[4], Svitlana P Tkachuk[3], Tetyana A Korolyshyn[4], Galyna Yo Matiyishyn[3], Walery Zukow[5], Igor L Popovych[4]

[1]Ukrainian Scientific Research Institute of Transport Medicine Ministry of Health, Odesa Gozhenko@mail.ru
[2]Hotel Spa Complex "Karpaty", Truskavets', Ukraine hrytsak.y@gmail.com
[3]JSC "Truskavets'kurort", Truskavets', Ukraine lgbarylyak@biph.kiev.ua
[4]Laboratory of Experimental Balneology, OO Bogomoletz Institute of Physiology NAS, Kyiv, Ukraine i.popovych@biph.kiev.ua; marta.kovbasnyuk@yandex.ua
[5]Faculty of Physical Education, Health and Tourism, Kazimierz Wielki University, Bydgoszcz, Poland w.zukow@ukw.edu.pl

Abstracts

Background. It is known that in practically healthy (without clinical diagnose) people constellations of principal Adaptation Hormones displays widely variability representing different gradations of health and premorbide states. Previously, we found at least four mutually separated groups of people (endocrine clusters). The **aim** of this study is to elucidate the characteristics of immunity in members of different neuroendocrine clusters. **Material and research methods.** We determined twice in ten women and ten men aged 33-76 years without clinical diagnose serum levels of Cortisol, Aldosterone, Triiodothyronine, Testosterone and Calcitonin (ELISA) as well as Bayevskiy's Stress Index by HRV as markers of General Adaptation Reactions (GAR). Also used determined by Leukocytogram Popovych's Strain and Adaptation Indices. Immune status evaluated on a set of I and II levels recommended by the WHO. **Results.** All 40 case was distributed among four neuroendocrine clusters. Cluster N1 contains 10% cases with drastically hypercortisolemia and increased Baevskiy's Stress Index combined with moderately decreased level of Testosterone but normal levels of Triiodothyronine, Aldosterone and Calcitonin. It is accompanied by a significant supression of cellular immunity and insignificant activation of humoral immunity. 12,5% cases (Cluster N2) characterized moderately increased level of Cortisol and Triiodothyronine combined with moderately decreased level of Calcitonin but normal levels of Testosterone and Aldosterone. Despite the normal average level of Stress Index it ranges in a wide range. This is accompanied by negligible inhibition of cellular immunity and significant activation of humoral immunity. 32,5% cases (Cluster N3) characterized moderately increased level of Cortisol too while moderately decreased level of Triiodothyronine as well as Calcitonin and Testosterone combined with upper borderline level of

Aldosterone and normal level of Stress Index. This is accompanied by a significant activation of humoral immunity without abnormalities of cellular immunity. In 45% cases (Cluster N4) neuro-hormonal costellation characterized upper borderline levels of Cortisol as well as Testosterone combined with normal levels others hormones and even slightly reduced level of Stress Index. This is accompanied by more pronounced activation of humoral immunity without abnormalities of cellular immunity. **Conclusion.** Various constellations of Neuroendocrine factors of General Adaptation Reactions accompanied by various constellations parameters of Immunity.
Keywords: Cortisol, Aldosterone, Triiodothyronine, Testosterone, Calcitonin, HRV, Immunity, Relationships.

INTRODUCTION

In the previous article and abstracts we [12,13,36] reported that in women and men aged 33-76 years without clinical diagnose constellations of principal Adaptation Hormones displays widely variability representing different gradations of health and premorbide states. All case was distributed among four clusters. Cluster N1 contains 10% cases with drastically hypercortisolemia and upper borderline level of Aldosterone reflected strong chronic stress; 20% cases characterized moderately increased level of Cortisol as well as Triiodothyronine and lower borderline level of Testosterone as well as Calcitonin, together reflected chronic stress too; 37,5% cases characterized upper borderline level of Cortisol and lower borderline level others three hormones, together reflected absence of stress, while in 32,5% cases hormonal costellation characterized upper borderline level of Cortisol as well as Testosterone and normal level others hormones, reflected absence of stress too. Estimation by Leukocytogram Popovych's Adaptation Index shown that at 62,5% cases take place Harmonious General Adaptation Reactions (GAR) as markers of health while at 37,5% cases detected Disharmonious GAR as markers of premorbidic stage. In this report we present received synchronously at the same cohort data about the parameters of autonomous regulation and immunity.

MATERIAL AND RESEARCH METHODS

Repeat that the study involved twentee volunteers – ten women and ten men aged 33-76 years without clinical diagnose. In the morning on an empty stomach in portion of capillary blood we counted up leukocytogram, on the basis of which determined Strain and Adaptation Indices according scale proposed IL Popovych [32] and approved a number of authors [4,18,27,28]. Then we recorded electrocardiogram in II lead (hardware-software complex "CardioLab+HRV" produced "KhAI-MEDICA", Kharkiv) to assess classic Bayevskiy's Stress Index by HRV [3].

In portion of venous blood determined hormonal and immune parameters.

Among hormones determined Cortisol, Aldosterone, Testosterone, Triiodothyronine and Calcitonin (by the ELISA with the use of analyzers "Tecan" and "RT-2100C" and corresponding sets of reagents from "Алкор Био", XEMA Co., Ltd and DRG International Inc.).

Immune status evaluated on a set of I and II levels recommended by the WHO as described in handbook [24]. 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. We carried out also test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of immunoglobulins classes G, A, M (ELISA, analysator "Immunochem", USA) and circulating immune complicis (with polyethylene glycol precipitation method).

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [9] with our (MM Kovbasnyuk) moderately modification. To do this, 5 drops of blood immediately after collection, made in glass centrifuge tubes with 2 ml of 4% solution of sodium citrate. Blood samples were stored in a refrigerator at a temperature of 4^0C. Further samples were centrifuged (5000 rev/min for 5 minutes). The supernatant was removed with the help of the Pasteur's pipette. We used a fraction of leukocytes with traces of erythrocytes. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-Positive Bacterias and Escherichia coli (O55 K59) as typical representative of Gram-Negative Bacterias. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". To prepare the suspension microbes did wipes with relevant shoals sterile saline, immersed tubes in boiling water for 3 seconds, cooled to room temperature. Integrity microbes controlled with the aid of a microscope. To do this, drop the suspension of microbes applied to skimmed substantive piece of glass, fixed in alcohol lamp flame. Ready preparations stained by Papenheim, microscoped during immersion, lense h90, eyepiece x10. The test samples were prepared as follows. In Vidal's plastic tubes made in the following order of 0,05 mL of heparin, 0,05 mL of sterile saline, 0,1 mL suspension of leukocytes, 0,05 mL suspension of microbial bodies. Samples shaked and placed in thermostat at 37^0C for 30 min, shaking them with every 10 minutes. Then, to stop phagocytosis, the sample was cooled under running water for 10 minutes. In further samples are centrifuged (5000 rev/min, for 5 minutes), the supernatant removed with the help of the Pasteur's pipette. From the suspension of leukocytes (with traces of red blood cells) prepared strokes, dried in air at room temperature and stained by Papenheim. Microscoped during immersion lens h90, x10 eyepiece.

Take into account the following parameters of phagocytosis: activity (percentage of neutrophils, in which found microbes - Phagocytic Index), intensity (number of microbes absorbed one phagocytes - Microbial Count) and completeness (percentage of dead microbes - Killing Index). Microbial number and index their digestion is determined for each phagocyte and fixed in phagocytic frame. As an integrated assessment of the phagocytic function of neutrophils considered the number of microbes that are able to neutralize neutrophils contained in 1 liter of blood, named as Bactericidity Capacity (BCC) and calculated by formula [18,21,34]:

BCC (10^9 Bac/L)=Leukocytes (10^9/L)•Neutrophils (%)•PhI (%)•MA (Bac/Phag)•KI (%)/10^6

After week all tests repeated.

Results processed using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

According recommendation by IL Popovych [34] neuroendocrine and 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 Neuroendocrine variables 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 [2,25].

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 [2,25].

We have identified 4 clusters, the composition of which is slightly different from those described previously, due to the additional inclusion of the factors of adaptation Stress index. The first cluster included the same 4 members, while the second cluster included others 5 members, the third 13, the fourth 18 (Table 1).

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

Cluster No 1	Case No. C_11	Case No. C_12	Case No. C_14	Case No. C_31
Distance	2,47	1,90	1,38	,88

Cluster No 2	Case No. C_1	Case No. C_3	Case No. C_18	Case No. C_23	Case No. C_38
Distance	1,40	1,50	,82	1,04	1,11

Cluster No 3	Case No. C_4	Case No. C_17	Case No. C_21	Case No. C_24	Case No. C_25	Case No. C_27	Case No. C_30	Case No. C_32	Case No. C_34	Case No. C_35	Case No. C_36	Case No. C_37	Case No. C_39
Distance	1,15	1,60	1,82	,75	,89	,95	,64	,95	,88	,71	,55	,85	,74

Cluster No 4	Case No. C_2	Case No. C_5	Case No. C_6	Case No. C_7	Case No. C_8	Case No. C_9	Case No. C_10	Case No. C_13	Case No. C_15	Case No. C_16	Case No. C_19	Case No. C_20	Case No. C_22
Distance	1,13	,66	,75	,60	,74	,80	,87	,48	1,01	,93	1,57	,85	,88

Cluster No4 (cont)	Case No. C_26	Case No. C_28	Case No. C_29	Case No. C_33	Case No. C_40
Distance	,66	1,2	1,2	1,0	1,0

Clusters appeared clearly delineated, as evidenced by the ratio between Distances from Respective Cluster Center (Table 1) and Euclidean Distances between Clusters (Table 2).

Table 2. Euclidean Distances between Clusters

Clusters	No. 1	No. 2	No. 3	No. 4
No. 1	0,00			
No. 2	3,59	0,00		
No. 3	3,69	2,04	0,00	
No. 4	4,09	1,91	1,16	0,00

In the third stage carried Analysis of Variance and ranking variables for coefficient η^2:

$\eta^2=Sb^2/(Sb^2+Sw^2)$,
$R=\eta$,
$F=[Sb^2(n-k)]/[Sw^2(k-1)]$, where
Sb^2 is Between Variance;
Sw^2 is Within Variance;
n is number of persons (40);
k is number of groups-clusters (4).

In our fun maximum coefficient η^2, is the largest contribution to the division into clusters, stated for Bayevskiy's Stress Index as integrated marker of sympathetic, vagal and humoral outflows on heart rate [3] as well as on immune organs [21,33,37,40,50,52]. Instead, do not play a significant role in clustering Calcitonin and Aldosterone (Table 3).

Table 3. Analysis of Variance

Variables	Between SS	Within SS	η^2	R	F	signif. p
Stress Index	241,3	78,6	0,754	0,869	36,8	10^{-6}
Triiodothyronine	89,7	39,2	0,696	0,834	27,5	10^{-6}
Cortisol	98,5	98,0	0,501	0,708	12,1	10^{-4}
Testosterone	26,0	32,7	0,443	0,666	9,5	10^{-4}
Calcitonin	5,08	31,8	0,138	0,371	1,9	,14
Aldosterone	1,97	16,1	0,109	0,330	1,5	,24

Specifications Neuroendocrine clusters displayed in the Table 4 and Figure 1. Cluster N1 contains 10% cases with drastically hypercortisolemia and increased Baevskiy's Stress Index combined with moderately decrease level of Testosterone but normal levels of Triiodothyronine, Aldosterone and Calcitonin. 12,5% cases (Cluster N2) characterized moderately increased level of Cortisol and Triiodothyronine combined with moderately decrease level of Calcitonin but normal levels of Testosterone and Aldosterone. Despite the normal average level of Stress Index it ranges in a wide range. 32,5% cases (Cluster N3) characterized moderately increased level of Cortisol too while moderately decrease level of Triiodothyronine as well as Calcitonin and Testosterone combined with upper borderline level of Aldosterone and normal level of Stress Index. In 45% cases (Cluster N4) neuro-hormonal costellation characterized upper borderline levels of Cortisol as well as Testosterone combined with normal levels others hormones and even slightly reduced level of Stress Index.

Table 4. Summary Cluster Analysis of Neuroendocrine variables (Mean±SE)

Variables (Mean Norm for females and males)	**Cluster N1 (n=4)**	**Cluster N2 (n=5)**	**Cluster N3 (n=13)**	**Cluster N4 (n=18)**
	Z-score=(Portio of Norm - 1)/Coefficient of Variation			
Cortisol (405 nM/L)	+6,10±1,63*	+2,47±0,50*	+2,78±0,51*	+0,85±0,25*
Stress Index HRV (139 un)	+7,85±1,72*	+0,55±1,06	-0,27±0,25	-0,49±0,18*
Triiodothyronine (2,20 nM/L)	+0,69±0,44	+3,77±0,73*	-1,10±0,22*	-0,28±0,24
Calcitonin (5,5; 13,95 ng/L)	+0,17±0,37	-0,96±0,17*	-0,82±0,21*	-0,28±0,27
Testosterone (2,3; 25,2 nM/L)	-0,72±0,35*	-0,43±0,44	-1,03±0,25*	+0,74±0,24*
Aldosterone (238 pM/L)	-0,21±0,32	+0,21±0,17	+0,50±0,19*	+0,12±0,17

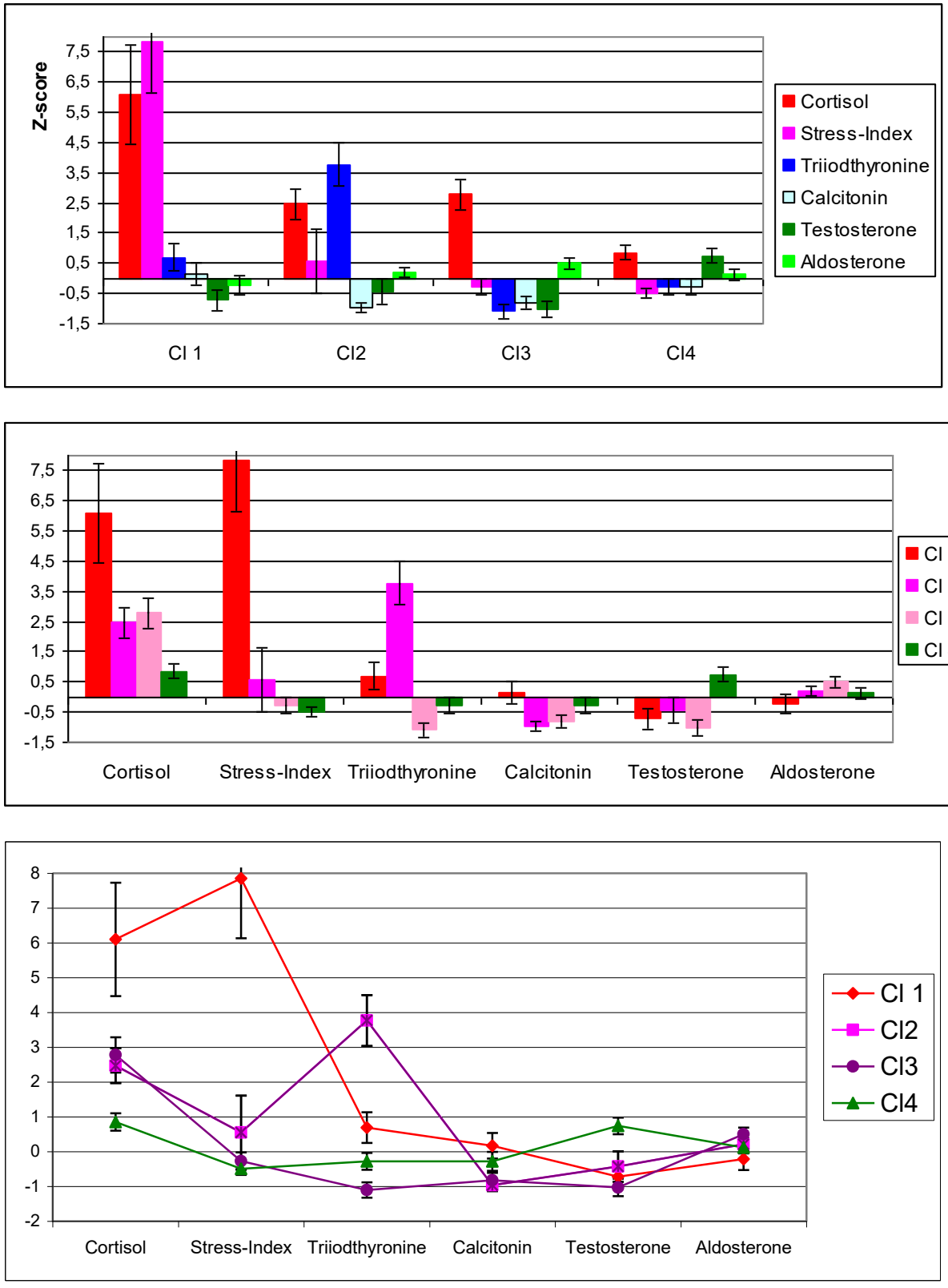


Fig. 1. Neuroendocrine profiles of various clusters

Now consider the immune support certain neuroendocrine constellations. As seen in Table 5 and Fig. 2, members Cluster N1 characterized by the following features of immune image: a significant reduction of T-helpers and Bacterocidity of Neutrophils as well as moderately decreased level of Circulating Immune Complicis and T-killers combined with normal levels of Natural Killers as well as B- and "Active" T-Lymphocytes. In return level of Immunoglobulines revealed increased. In circulating blood there are 0-Lymphocytes, that normally are not available, that is immature and/or damaged. Cellular Immunity Index calculated as average Z-scores for 6 parameters ($CD4^+$, $CD8^+$, $CD16^+$ and "Active" T-Lymphocytes as well as Bacterocidity of Neutrophils) is much undervalued. Consequently, there is pronounced suppression of cellular immunity. In return Humoral Immunity Index calculated as average Z-scores for 5 parameters (B-Lymphocytes, Immunoglobulines and CIC) is moderately elevated, indicating moderately activation of humoral immunity. Integrated Immunity Index calculated as average Z-scores of 11 immune parameters is still moderately undervalued. This neuroendocrine-immune image we assess how heavy stress (**distress**) described by many authors [5,8,10,11,14,16,17,34,35,41,51].

On the other hand, Popovych's Strain Index (PSI) calculated by formula [18]:
$PSI=[(Leukocytes/6\text{-}1)^2+(Stub\ Neutrophils/3{,}5\text{-}1)^2+(Eosinophils/3{,}5\text{-}1)^2+(Monocytes/5{,}5\text{-}1)^2]/4$
is drastically increased while Popovych's Leukocytogram Adaptation Index is twice decreased indicating **significant dysadaptation**.

Table 5. Features of the immune status of the different Neuro-Hormonal clusters (Mean±SE)

Variables (Mean Norm)	**Cluster N1 (n=4)**	**Cluster N2 (n=5)**	**Cluster N3 (n=13)**	**Cluster N4 (n=18)**
	Z-score=(Portio of Norm - 1)/Coefficient of Variation			
$CD4^+$ T-Lymphocytes (39,5 %)	-2,16±0,15*	-0,83±0,68	-1,22±0,39*	0,00±0,38
Bacterocidity vs E. coli ($95•10^9$ Micr/L)	-2,15±0,66*	-0,87±0,92	+0,47±0,47	-0,18±0,53
Bacterocidity vs St. aur. ($102•10^9$ Micr/L)	-1,80±1,25	-0,98±0,63	-0,49±0,58	-0,44±0,29
$CD8^+$ T-Lymphocytes (23,5 %)	-0,77±0,38	-0,22±0,93	+0,06±0,40	+0,33±0,28
"Active" T-Lymphocytes (30 %)	0,00±0,66	-0,12±0,37	+0,23±0,22	+0,31±0,19
$CD16^+$ NK-Lymphocytes (17 %)	+0,05±0,32	+0,49±0,48	+0,53±0,60	+1,62±0,31*
$CD22^+$ B-Lymphocytes (20 %)	+0,43±0,58	+0,23±0,41	+0,64±0,36	+1,41±0,26*
Immunoglobulines G (12,75 g/L)	+0,61±0,27*	+0,64±0,33	+1,11±0,40*	+1,32±0,31*
Immunoglobulines A (1,875 g/L)	+0,77±0,25*	+0,80±0,46	+0,68±0,22*	+1,01±0,11*
Immunoglobulines M (1,15 g/L)	+1,06±0,56	+1,63±0,30*	+1,14±0,20*	+1,39±0,21*
Circulating Immune Complex (45 un)	-0,99±0,21*	+0,37±0,43	-0,41±0,19*	-0,60±0,16*
Cellular Immunity Index (0)	-1,14±0,34*	-0,42±0,46	-0,07±0,33	+0,27±0,19
Humoral Immunity Index (0)	+0,38±0,20	+0,73±0,13*	+0,63±0,16*	+0,91±0,12*
Integrated Immunity Index (0)	-0,45±0,13*	+0,10±0,28	+0,25±0,21	+0,56±0,09*
	Mean±Standard Error			
0-Lymphocytes (0 %)	7,9±2,1*	1,2±6,0	0,0±4,4	-10,7±2,0*
Popovych's LCG Strain Index (0,09)	0,73±0,32*	0,16±0,03	0,15±0,03	0,09±0,02
Popovych's LCG Adaptation Index (1,70)	0,86±0,07*	1,37±0,18	1,31±0,15	1,45±0,09

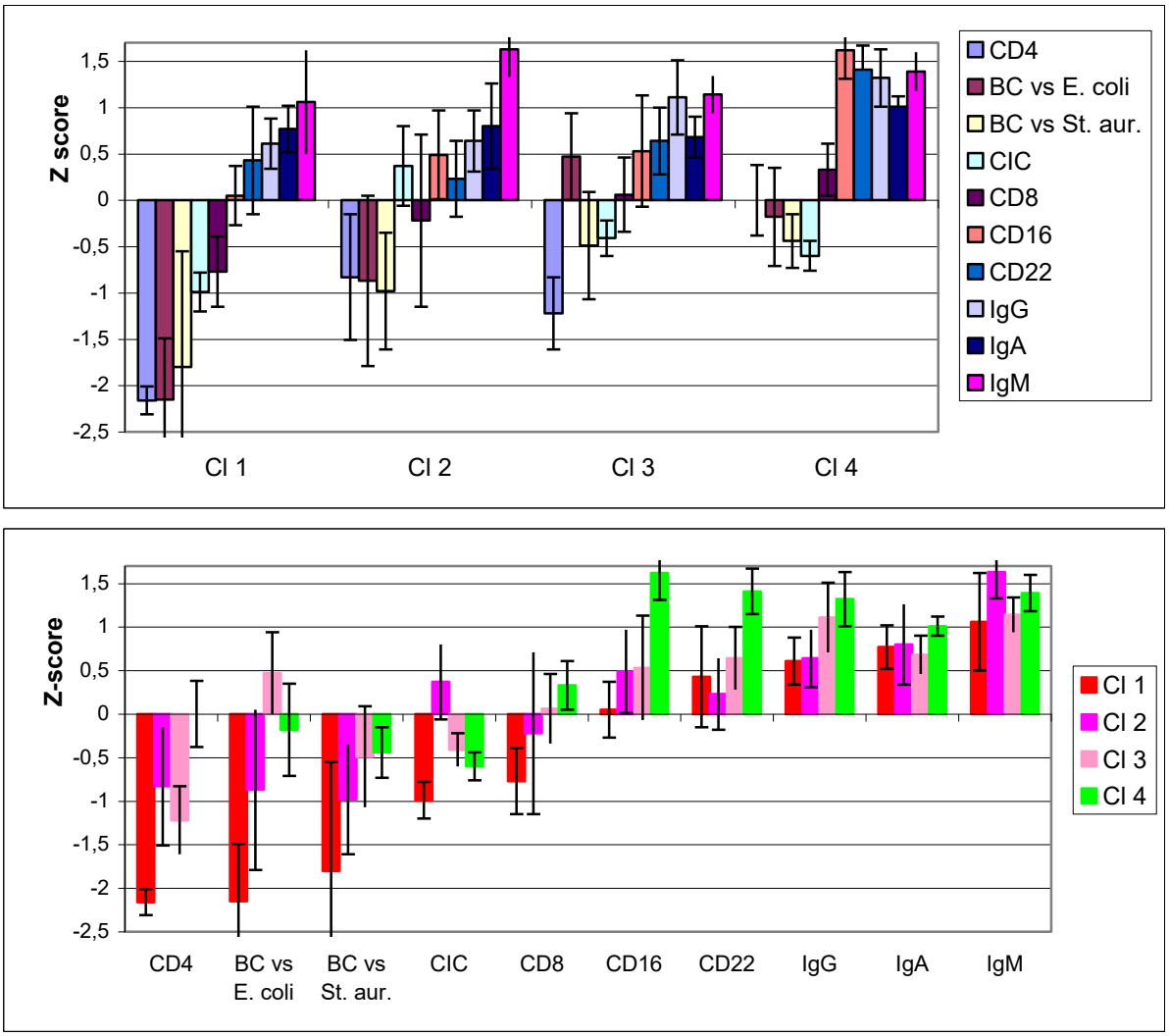


Fig. 2. Immune profiles of various clusters

Members Cluster N2 characterized by moderately reduction of T-helpers and Bacterocidity of Neutrophils combined with normal levels other indicators of Cellular Immunity. Content 0-Lymphocytes falls to a minimum. As a result, Cellular Immunity Index is reduced slightly. Instead decresed level CIC reverses and level IgA increases even more, so the Humoral Immunity Index much rises. Described Neuroendocrine-immune constellation in principle characteristic of **moderate stress** [5,8,19,20,22,29-31,46,47,52]. Popovych's Strain Index as well as Adaptation Index only slightly different from normal indicating **moderate dysadaptation**.

Members Cluster N3 characterized by normal levels indicators of Cellular Immunity (except T-helpers) combined with increased Humoral Immunity Index through the hight level IgG and despite the low level CIC. Both Popovych's indexes also remain at the previous cluster. This allows us to assess the state of Neuroendocrine-immune constellation as **moderate stress and dysadaptation** well, but closer to the norm.

Members Cluster N4 characterized by normal levels indicators of Cellular Immunity well, in addition, they have significantly elevated level of Natural Killers as well as B-Lymphocytes. The level of 0-lymphocytes, calculated by balance method, indicated a negative number, indicating that the presence of so-called D-Lymphocytes that express receptors characteristic of two

populations, probably B and NK or T? Levels of Immunoglobulines reaching highs for the cohort that despite reduced levels of CIC gives more perceptible rise the Humoral Immunity Index. Popovych's Strain Index equals the average norm, and Popovych's Adaptation Index is in its lower zone. Above, taking into account the neuroendocrine status allows us to characterize the state of the people of this cluster as **normal** or even optimal norm.

In order to detect immune parameters, which set of Neuro-Hormonal clusters different from each other, discriminant analysis was conducted (method forward stepwise [15]). The program is included in the model 7 immune parameters and Popovych's Leukocytogram Strain Index (Tables 6 and 7).

Identification information is condensed in three canonical discriminant roots (Table 8). The first Root contains 56,3% discriminant properties and displays, according to the structural coefficients, levels of Baevskiy's Stress Index, Cortisol and Popovych's Strain Index by **direct** way while parameters of Cellular Immunity by **inverse** way.

Table 6. Discriminant Function Analysis Summary. Recognized Variables for various clusters

Step 14, N of vars in model: 14; Grouping: 4 grps

Wilks' Lambda: 0,003; approx. $F_{(43)}=10,3$; $p<10^{-6}$

Variables currently in the model (Mean Norm)	Neuro-Hormonal Clusters (n)				Wilks Λ	Part. Λ	F-remove	p-level	Tolerancy
	I (4)	II (5)	III (13)	IV (18)					
Baevskiy's HRV Stress Index, σ units (139)	+7,85 663	+0,55 169	-0,27 131	-0,49 104	,011	,255	22,4	10^{-6}	,202
Cortisol, σ nM/L (405)	+6,10 1183	+2,47 720	+2,78 760	+0,85 513	,007	,422	10,5	10^{-3}	,217
Popovych's Leukocytogram Strain Index, units (0,09)	- 0,73	- 0,16	- 0,15	- 0,09	,005	,600	5,1	,007	,135
$CD4^+$ T-Lymphocytes, σ % (39,5)	-2,16 32,5	-0,83 36,8	-1,22 35,5	0,00 39,5	,005	,594	5,2	,007	,205
$CD22^+$ B-Lymphocytes, σ % (20,0)	+0,43 21,5	+0,23 20,8	+0,64 22,2	+1,41 24,9	,004	,773	2,3	,109	,457
$CD16^+$ NK-Lymphocytes, σ % (17,0)	+0,05 17,2	+0,49 18,4	+0,53 18,6	+1,62 21,7	,003	,818	1,7	,192	,185
$CD8^+$ T-Lymphocytes, σ % (23,5)	-0,77 21,0	-0,22 22,8	+0,06 23,7	+0,33 24,6	,004	,701	3,3	,039	,276
Testosterone, σ Females, nM/L (2,30) Males, nM/L (25,2)	-0,72 2,12 7,5	-0,43 2,62 11,9	-1,03 2,21 7,8	+0,74 3,11 15,0	,006	,443	9,6	10^{-3}	,473
Calcitonin, σ Females, ng/L (5,5) Males, ng/L (13,95)	+0,17 7,5 11,1	-0,96 3,3 6,7	-0,82 4,8 6,7	-0,28 5,7 7,4	,004	,707	3,2	,043	,699
Immunoglobulines A, σ g/L (1,875)	+0,77 2,12	+0,80 2,13	+0,68 2,09	+1,01 2,19	,004	,736	2,8	,065	,271
Aldosterone, σ pM/L (238)	-0,21 233	+0,21 243	+0,50 250	+0,12 241	,003	,792	2,0	,140	,700
Circulating Immune Complex, σ un (45)	-0,99 28	+0,37 51	-0,41 38	-0,60 34	,004	,681	3,6	,029	,502
Triiodothyronine, σ nM/L (2,20)	+0,69 2,55	+3,77 4,08	-1,10 1,65	-0,28 2,06	,011	,254	22,5	10^{-6}	,617
Immunoglobulines M, σ g/L (1,15)	+1,06 1,44	+1,63 1,60	+1,14 1,46	+1,39 1,53	,003	,878	1,1	,382	,595

Variables currently not in the model	Neuro-Hormonal Clusters				Wilks' Λ	Parti-al Λ	F to enter	p-le-vel	Tole-rancy
	I (4)	II (5)	III (13)	IV (18)					
Bactericidity vs Staph. aur., σ 10^9 Microbes/L (102)	-1,80 83	-0,98 92	-0,49 97	-0,44 97	,003	,905	,77	,52	,609
Bactericidity versus E. coli, σ 10^9 Microbes/L (95)	-2,15 75	-0,87 87	+0,47 99	-0,18 93	,003	,970	,22	,88	,721
Immunoglobulines G, σ g/L (12,75)	+0,61 14,3	+0,64 14,4	+1,11 15,7	+1,32 16,2	,002	,889	,91	,45	,457
"Active" T-Lymphocytes, σ % (30,0)	0,00 30,0	-0,12 29,4	+0,23 31,2	+0,31 31,6	,003	,919	,65	,59	,631
0-Lymphocytes, % (0,0)	- 7,9	- 1,2	- 0,0	- -10,7	,002	,889	,91	,45	,457
Popovych's Leukocytogram Adaptation Index (1,46÷1,95)	- 0,86	- 1,37	- 1,31	- 1,45	,003	,974	,20	,90	,317

Table 7. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Baevskiy's Stress Index	36,8	10^{-6}	,246	36,8	10^{-6}
Triiodothyronine	30,4	10^{-6}	,068	33,0	10^{-6}
Testosterone	8,5	10^{-3}	,039	25,8	10^{-6}
Cortisol	6,6	,001	,024	22,5	10^{-6}
$CD4^+$ T-Lymphocytes	3,6	,024	,018	19,4	10^{-6}
Circulating Immune Complex	3,6	,024	,013	17,6	10^{-6}
Calcitonin	3,0	,046	,010	16,2	10^{-6}
Popovych's Strain Index	2,0	,130	,009	14,7	10^{-6}
Aldosterone	2,2	,111	,007	13,7	10^{-6}
Immunoglobulines M	1,7	,200	,006	12,7	10^{-6}
Immunoglobulines A	1,2	,346	,005	11,7	10^{-6}
$CD8^+$ T-Lymphocytes	2,1	,132	,004	11,2	10^{-6}
$CD22^+$ B-Lymphocytes	1,8	,177	,003	10,7	10^{-6}
$CD16^+$ NK-Lymphocytes	1,7	,193	,003	10,3	10^{-6}

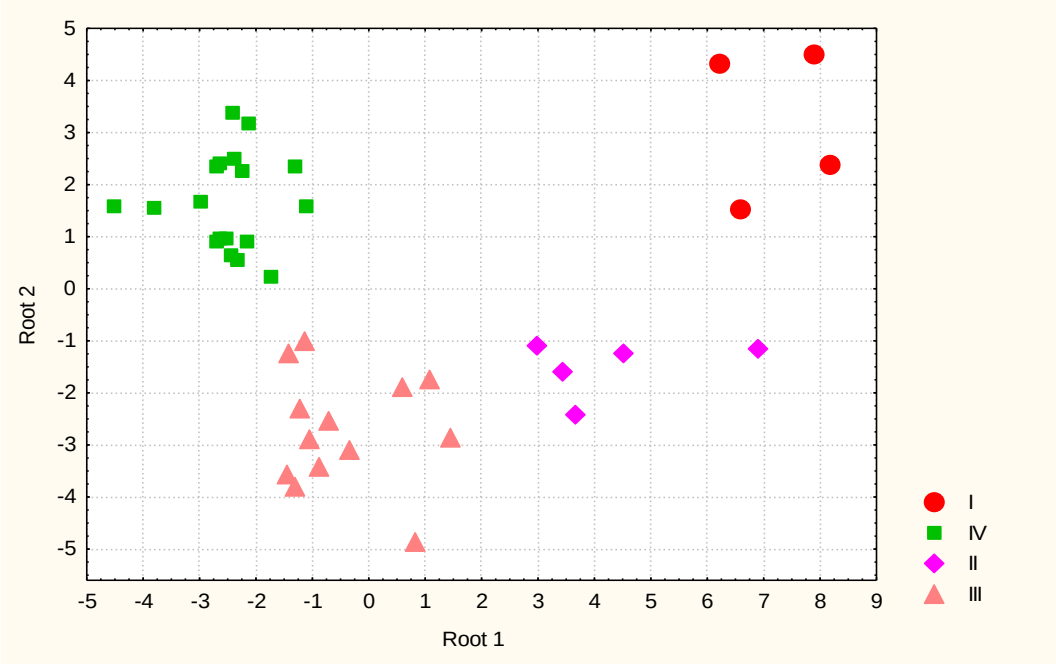
Table 8. Standardized, Structural and Raw Coefficients and Constants for Canonical Variables

Variables currently in the model	Standardized			Structural			Raw		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Baevskiy's Stress Index	1,609	1,199	-,351	**,43**	,31	-,36	1,089	,811	-,237
Cortisol	1,409	-,757	-,667	**,26**	-,04	-,26	,854	-,459	-,404
Popovych's Strain Index	-1,791	-,018	,150	**,23**	,14	-,24	-8,80	-,089	,739
$CD4^+$ T-Lymphocytes	-,597	1,392	-,146	**-,12**	,08	,15	-,409	,954	-,100
$CD22^+$ B-Lymphocytes	-,466	,584	-,106	**-,10**	,10	,00	-,399	,500	-,091
$CD16^+$ NK-Lymphocytes	,027	-1,040	,306	**-,10**	,09	,06	,016	-,611	,180
$CD8^+$ T-Lymphocytes	,336	,904	-,610	**-,07**	-,00	,03	,245	,657	-,443
Testosterone	-,552	,997	,271	-,15	**,28**	,19	-,578	1,045	,284
Calcitonin	-,208	,661	,110	,00	**,16**	-,07	-,221	,704	,117
Immunoglobulines A	,972	-,138	-,341	-,03	**,08**	,05	1,451	-,206	-,510
Aldosterone	-,216	-,547	,029	-,04	**-,14**	-,02	-,323	-,818	,043
Circulating Immune Complex	,510	-,529	,453	,03	**-,14**	,22	,720	-,746	,639
Triiodothyronine	,752	,052	,940	,28	,05	**,64**	,721	,050	,901

Immunoglobulines M	,378	-,034	,306	-,01	,02	**,11**	,446	-,040	,360
Eigenvalues	11,51	5,48	3,46			**Constants**	-3,03	2,49	1,06
Canonical R	,959	,912	,881			**Discriminant Prop, %**	56,3	26,8	16,9
Wilks' Lambda	,003	,035	,224			Means of Roots Cl IV	-2,49	1,67	0,44
Chi-Square	177	101	45			Means of Roots Cl III	-0,43	-2,71	-1,33
Degree Freedom	42	26	12			Means of Roots Cl II	4,30	-1,50	3,85
p-level	10^{-6}	10^{-6}	10^{-4}			Means of Roots Cl I	7,22	3,18	-2,45

The second Root contains 26,8% discriminant properties and reflects by **direct** way levels of Testosterone, Calcitonin and Immunoglobulines A while by **inverse** way levels of Aldosterone and CIC. The remaining 16,9% are third Root, which represents levels of Triiodothyronine and Immunoglobulines M (Table 8).

Calculation of Individual Roots values by summation products multiplying of individual Variables currently in the model by Raw Coefficients (plus Constsnts) allows as to visualize the location of each person on the plane discriminant roots (Fig. 3).



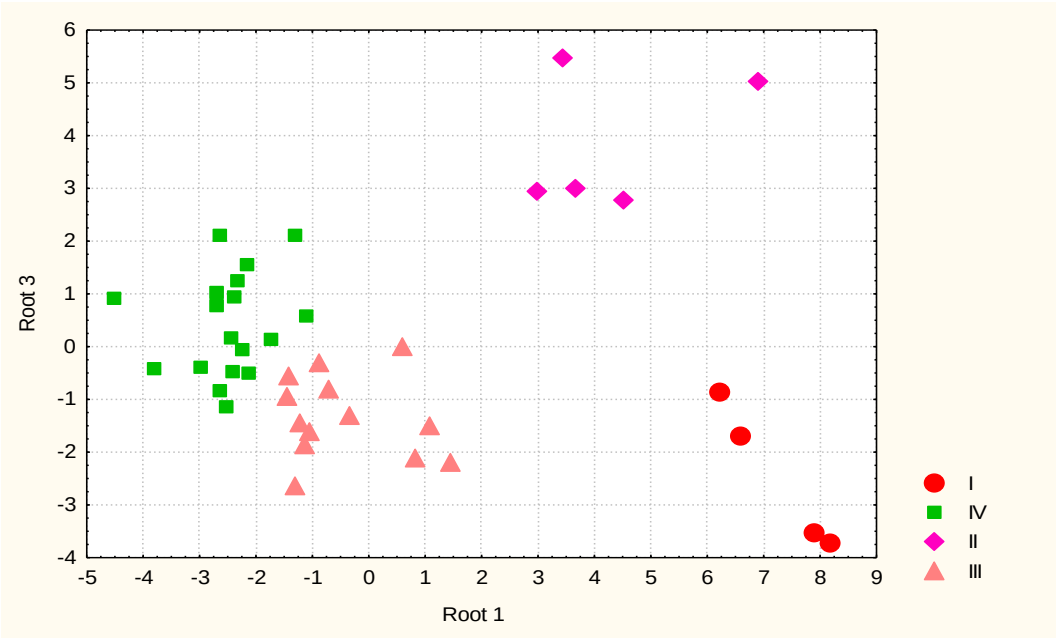


Fig 3. Individual Roots values for members of various clusters

Apparently, members of the IV cluster occupy the left area of the axis of the first root, reflecting minimal for cohort level Neuroendocrine Stress factors, combined with the highest levels of parameters of Cellular immunity, while members of I cluster located in the right area, which represents the maximum levels of Stress and suppression of Cellular immunity, in turn members of clusters II and III occupy intermediate positions.

On the other hand, localization members of II and III clusters in the lower zone of the second root represents the minimum for the cohort levels Testosterone, Calcitonin and Immunoglobulines A while maximum levels of Aldosterone and CIC.

Finally, the highest placement along the axis of the third root members of cluster II meet their maximum levels of Triiodothyronine and Immunoglobulines M.

In general, all clusters are very clearly delineated that documented statistics (Table 9).

Table 9. Squared Mahalanobis Distances between clusters and F-values (df=14); p-levels: $10^{-6} \div 10^{-4}$

Cluster	I	IV	II	III
I	0	116	78	105
IV	**13,5**	0	75	29
II	**6,1**	**11,1**	0	56
III	**11,5**	**9,5**	**7,7**	0

Calculation of Classification Functions for them Coefficients and Constants (Table 10) allows to accurately identify each member of the cohort (Table 11).

Table 10. Coefficients and Constants for Classification Functions

Variables (as Z-scores) currently in the model	IV	III	II	I
Baevskiy's Stress Index	,51	-,39	4,51	12,98
Triiodothyronine	,17	-,16	7,98	4,65

Testosterone	-2,44	-8,71	-8,71	-7,30
Cortisol	2,62	7,10	8,49	11,39
$CD4^+$ T-Lymphocytes	-2,33	-7,17	-8,47	-4,57
Circulating Immune Complex	-2,76	,86	6,67	1,26
Calcitonin	-1,09	-4,84	-4,43	-2,51
Aldosterone	1,75	4,60	2,31	-2,74
Immunoglobulines M	3,68	4,13	8,06	6,90
Immunoglobulines A	7,22	12,01	15,98	22,47
$CD8^+$ T-Lymphocytes	,58	-1,02	-1,36	5,22
$CD22^+$ B-Lymphocytes	-1,43	-4,28	-6,04	-4,29
$CD16^+$ NK-Lymphocytes	2,97	5,36	5,63	1,69
Popovych's Strain Index, un	-21,60	-40,59	-78,52	-109,3
Constants	-10,46	-29,86	-49,77	-70,18

Table 11. Classification Matrix. Rows: Observed classifications, Columns: Predicted classifications

Cluster	Percent correct	I	IV	II	III
		p=,100	p=,450	p=,125	p=,325
I	100	**4**	0	0	0
IV	100	0	**18**	0	0
II	100	0	0	**5**	0
III	100	0	0	0	**13**
Total	100	4	18	5	13

At the last stage maked canonical correlation between neuroendocrine parameters on one side and the parameters of immunity on the other.

According to calculations by the formula: $|r|=\{\exp[2t/(n-1,5)^{0,5}] - 1\}/\{\exp[2t/(n-1,5)^{0,5}]+1\}$ for a sample of n=40 critical value |r| at p<0,05 (t>2,02) is 0,31. Minimum |r| for inclusion in the canonical analysis is 0,25. For this reason, dropped from the analysis the level of NK- and "active" T-Lymphocytes as well as IgM and CIC.

As a factor signs we took 6 Neuroendocrine variables (Table 12).

Table 12. Correlations between Neuroendocrine variables expressed as Z-scores (right set)

Variables Expressed as Z-scores	Baevs-kiy's Stress Index	Cor-tisol	Testo-stero-ne	Aldo-stero-ne	Tri-iodo-thyro-nine	Cal-cito-nin
Baevskiy's Stress Index	1,00	**,60**	-,25	*-,05*	*,10*	*,19*
Cortisol	**,60**	1,00	*-,19*	*,09*	*,17*	*,19*
Testosterone	-,25	*-,19*	1,00	*-,08*	*,08*	*,08*
Aldosterone	*-,05*	*,09*	*-,08*	1,00	*-,12*	*-,06*
Triiodothyronine	*,10*	*,17*	*,08*	*-,12*	1,00	*-,07*
Calcitonin	*,19*	*,19*	*,08*	*-,06*	*-,07*	1,00

Instead indicators Immunity act as an effective signs (Table 13).

Table 13. Correlations between Immune variables expressed as Z-scores (left set)

Variables	0-L	BCA	BCE	Strain	Adapt	IntImI	$CD4^+$	$CD8^+$	$CD22^+$	IgG	IgA
0-Lymphocytes	1,00	**-,36**	-,28	**,33**	*-,03*	**-,89**	**-,81**	**-,58**	**-,49**	*-,03*	*-,10*

BC vs Staph aur	**-,36**	1,00	**,61**	**-,38**	*,17*	**,63**	,30	*,22*	*,17*	*-,22*	*-,15*
BC vs E. coli	-,28	**,61**	1,00	**-,33**	*,06*	**,58**	*,04*	**,47**	*,13*	**-,42**	*-,02*
Strain Index	**,33**	**-,38**	**-,33**	1,00	**-,45**	**-,46**	-,25	-,27	-,25	*-,10*	*,03*
Adaptation Ind	*-,03*	*,17*	*,06*	**-,45**	1,00	*,20*	*,02*	*-,01*	*,10*	,29	*,12*
Integra Imm Ind	**-,89**	**,63**	**,58**	**-,46**	*,20*	1,00	**,67**	**,54**	**,49**	*,04*	*,09*
$CD4^+$	**-,81**	,30	,04	-,25	*,02*	**,67**	1,00	*,19*	,24	*,06*	*-,02*
$CD8^+$	**-,58**	*,22*	**,47**	-,27	*-,01*	**,54**	*,19*	1,00	*-,04*	-,25	*-,17*
$CD22^+$	**-,49**	*,17*	*,13*	-,25	*,10*	**,49**	,24	*-,04*	1,00	,24	**,50**
IgG	*-,03*	*-,22*	**-,42**	*-,10*	,29	*,04*	*,06*	-,25	,24	1,00	*,09*
IgA	*-,10*	*-,15*	*-,02*	*,03*	*,12*	*,09*	*-,02*	*-,17*	**,50**	*,09*	1,00

This division concerning causation entirely conditional insofar as the nervous, endocrine and immune systems are interconnected [1,16,17,21,33,34,35,51]. Correlation Matrix (Table 14) confirms maximal Immunotropic activity ANS and Cortisol [5-8,42-45,48-51].

Table 14. Correlations between Neuroendocrine and Immune variables

Variables Expressed as Z-scores	Baevs-kiy's Stress Index	Cor-tisol	Testo-stero-ne	Aldo-stero-ne	Tri-iodo-thyro-nine	Cal-cito-nin
$CD4^+$ T-Lymphocytes	-,29	-,25	*,08*	*,05*	*-,01*	*-,05*
$CD8^+$ T-Lymphocytes	**-,35**	-,28	*,05*	*-,01*	*-,03*	*,01*
$CD22^+$ B-Lymphocytes	-,28	*-,15*	**,36**	*-,08*	*-,09*	*,02*
0-Lymphocytes	**,39**	**,33**	*-,22*	*,04*	*,09*	*-,03*
Immunoglobulines G	*-,07*	*-,20*	*,24*	,25	-,26	-,26
Immunoglobulines A	*-,18*	*-,16*	**,38**	**-,31**	*,11*	*-,00*
Bactericidity vs Staph. aureus	*-,20*	-,27	*-,12*	*-,03*	*-,15*	*-,08*
Bactericidity vs E. coli	**-,39**	*-,20*	*-,08*	*,01*	*-,15*	*-,07*
Integrated Immunity Index	**-,48**	**-,42**	*,16*	*,04*	*-,16*	*-,08*
Popovych's LCG Strain Index	**,82**	**,76**	*-,13*	*-,02*	*,14*	*,22*
Popovych's LCG Adaptation Index	-,29	**-,69**	*-,02*	*,20*	*-,17*	*-,14*

The program identified two pairs of canonical roots. Neuroendocrine root first pair receives a positive factor loading of Cortisol, Baevskiy's Stress Index and Calcitonin while immune root represents straight Popovych's Strain Index and 0-Lymphocytes and inverse way Popovych's Adaptation Index, Integrated Immunity Index as well as Bactericidity and subpopulations of T-Lymphocytes (Table 15). Relationship is very strong (Fig. 4).

Table 15. Factor Structure for Causal and Effective Variables

Variables	Root 1	Root 2
Right set (X-line)		
Cortisol	**,89**	,24
Baevskiy's Stress Index	**,88**	-,40
Calcitonin	**,28**	,08
Aldosterone	-,07	**-,48**
Testosterone	-,18	**,41**
Triiodothyronine	,15	**,29**
Left set (Y-line)		
Popovych's Strain Index	**,92**	-,10
0-Lymphocytes	**,40**	-,19
Popovych's Adaptation Index	**-,61**	**-,65**

Integrated Immunity Index	**-,52**	,08
$CD8^+$ T-Lymphocytes	**-,36**	,12
Bactericidity vs E. coli	**-,34**	,16
$CD4^+$ T-Lymphocytes	**-,32**	,04
Bactericidity vs Staph. aur.	**-,28**	-,12
Immunoglobulines A	-,14	**,44**
$CD22^+$ B-Lymphocytes	-,22	**,36**
Immunoglobulines G	-,19	**-,32**
Canonical R	0,959	0,739
Canonical R^2	0,919	0,546
Chi-square	132	56
Degree of freedom	66	50
p-level	10^{-5}	0,25
Lambda Prime	0,012	0,154

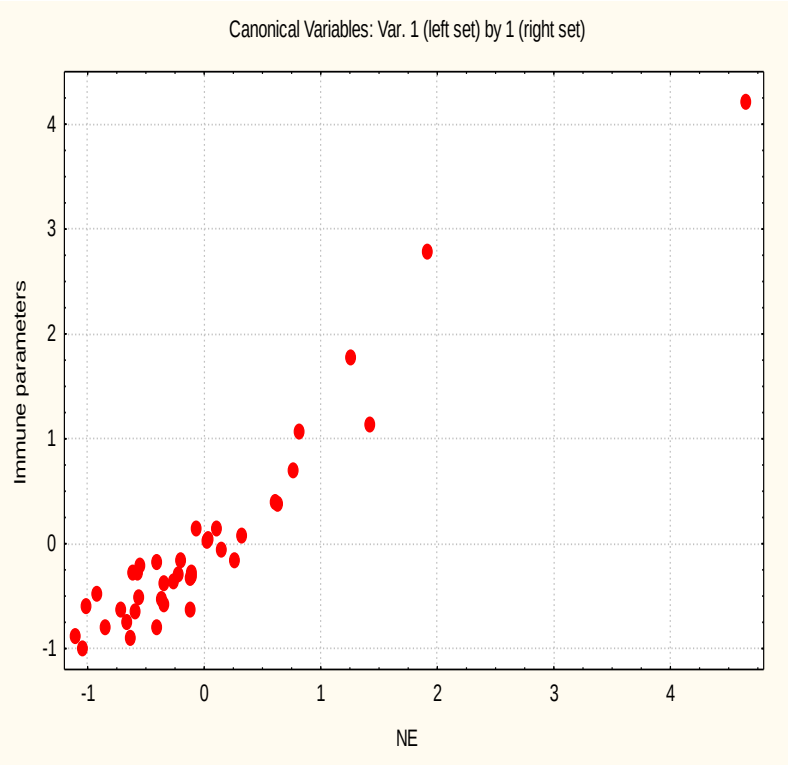


Fig 4. Canonical correlation between first pair of Neuroendocrine (X-line) and Immune (Y-line) variables

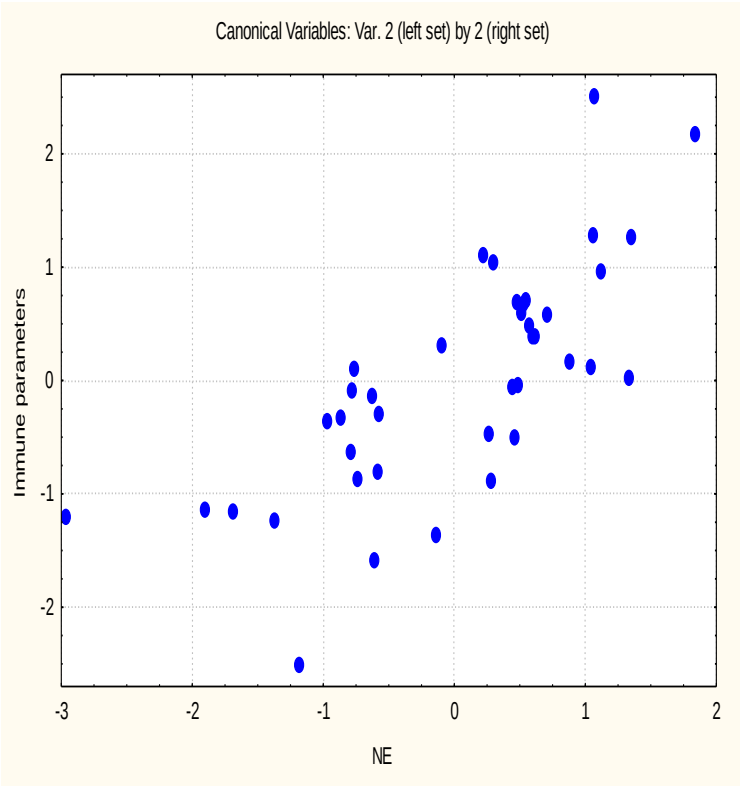


Fig 5. Canonical correlation between second pair of Neuroendocrine (X-line) and Immune (Y-line) variables

Neuroendocrine root second pair receives a positive factor loading of Testosterone and Triiodothyronine while negative loading of Baevskiy's Stress Index and Aldosterone. Immune root represents straight B-Lymphocytes and IgA while inverse way Popovych's Adaptation Index and IgG (Table 15). Relationship is less than for first pair but very strong too (Fig. 5).

As seen in the correlation matrix (Table 14) Neutrophils Bactericidity against E. coli downregulated by Sympathetic outflows while against Staph. aureus by Cortisol. On the other hand, Bactericidity against E. coli upregulated by T-killers and downregulated by IgG while against Staph. aureus upregulated by T-helpers also slightly by T-killers (Table 13) as well as by NK (r=0,31). Previously, we found differences in the nervous regulation of Neutrophils Bactericidity against E. coli and Staph. aureus in patients with chronic diseases [23,38,39]. Our findings are consistent with data RH Straub et al [44,45] that prior ablation of the Sympathetic nervous system decreases dissemination of E. coli through a mechanism of improved phagocytic response of peritoneal macrophages but contrary to this an increase dissemination of Staph. aureus, which was caused by reduction in corticosterone tonus.

This issue will focus on our next publication.

ACKNOWLEDGMENT

We express our sincere gratitude to administration JSC "Truskavets'kurort" for help in conducting immune tests and recording HRV.

ACCORDANCE TO ETHICS STANDARDS

Tests in participants 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. Akmayev PG. Modern understanding of the interactions of regulatory systems: the nervous, endocrine and immune [in Russian]. Uspekhi fiziologicheskikh nauk. 1996; 27(1): 3-20.
2. 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.
3. Baevskiy RM, Ivanov GG. Heart Rate Variability: theoretical aspects and possibilities of clinical application [in Russian]. Ultrazvukovaya i funktsionalnaya diagnostika. 2001; 3: 106-127.
4. 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.
5. Chrousos GP. The stress response and immune function: clinical implications. The 1999 Novera H Spector lecture. Neuroimmunomodulation. Perspectives at the new millenium. ANYAS. 2000; 917: 38-67.
6. Czura SJ, Tracey KJ. Autonomic neural regulation of immunity. J Intern Med. 2005; 257(2): 156-166.
7. Denyssenko PP. Role of Cholinereactive Systems in Processes of Regulation [in Russian]. Moskwa: Meditsina. 1980. 296 p.
8. Dhabhar FS. Enhancing versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection and Immunopathology. Neuroimmunomodulation. 2009; 16(5): 300–317.
9. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil. 1981. 110 p.
10. Garkavi LKh, Kvakina YeB, Kuz'menko TS. Antistress Reactions and Activation Therapy [in Russian]. Moskva: Imedis, 1998. 654 p.
11. Garkavi LKh, Kvakina EB, Ukolova MA. Adaptation Reactions and Resistance of Organism [in Russian]. Rostov na Donu: Publishing House of Rostov University Press, 3rd ed. 1990. 224 p.
12. Hrytsak YaL, Barylyak LG, Zukow W, Popovych IL. Cluster analysis of hormonal constellation at women and men with harmonious and disharmonious general adaptation reactions. Journal of Education, Health and Sport. 2016; 6(4): 141-150.
13. Hrytsak YaL, Barylyak LG, Kovbasnyuk MM, Popovych IL. Plasma levels principal adaptation hormones and parameters of immunity closely interact. In: Valeology: current status, trends and persrectives of development. Abstracts. XIV Intern. scient. and practical conf. (Kharkiv-Drohobych, 14-16 April 2016). Kharkiv: VN Karazin KhNU. 2016: 354-356.
14. Khaitov RM. Physiology of the Immune System [in Russian]. Moskwa: VINITI RAS. 2005. 428 p.
15. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika. 1989: 78-138.
16. Kolyada TI, Volyans'kyi YuL, Vasilyev NV, Mal'tsev VI. Adaptation Syndrome and Immunity [in Russian]. Kharkiv: Osnova. 1995. 368 p.
17. Korneva EA (editor). Immunophysiology [in Russian]. St-Pb: Nauka. 1993. 684 p.
18. Kostyuk PG, Popovych IL, Ivassivka SV, Chebanenko LO, Kyjenko VM (editors). Chornobyl', Adaptive and Defensive Systems, Rehabilitation [in Ukrainian]. Kyiv: Computerpress. 2006. 348 p.
19. Kozyavkina OV. Vegetotropic effects of bioactive water Naftyssya at children with dysfunction of the neuroendocrine-immune complex, these endocrine-immune accompaniment and the ability to predict [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 24-39.
20. Kozyavkina OV. Vegetotropic effects of bioactive water Naftyssya at males rats and their endocrine, electrolyte iand mmune accompaniments [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(3): 65-92.
21. 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.
22. Kozyavkina OV, Popovych IL, Zukow W. Immediate vegetotropic effects of bioactive water Naftussya and their neuro-endocrine-immune accompaniment in healthy men. Journal of Health Sciences. 2013; 3(5): 391-408.
23. Kul'chyns'kyi AB. The relationships between changes in parameters of Electroencephalogram and Immunogram in the conditions balneotherapy on spa Truskavets' [in Ukrainian]. In: Valeology: current status, trends and persrectives of development. Abstracts. XIV Intern. scient. and practical. conf. (Kharkiv-Drohobych,

14-16 April 2016). Kharkiv: VN Karazin KhNU. 2016: 322-323.
24. Lapovets' LYe, Lutsyk BD. Handbook of Laboratory Immunology [in Ukrainian]. L'viv. 2002. 173 p.
25. Mandel ID. Cluster analysis [trans. from English in Russian]. Moskva: Finansy i Statistika. 1988. 176 p.
26. Nance DM, Sanders VM. Autonomic innervation and regulation of immune system (1987-2007). Brain Behav Immun. 2007; 21(6): 736-745.
27. Petsyukh SV, Petsyukh MS. Leukocytary Popovych's adaptation index correlated with parameters of ongoiging HRV and EEG. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 19.
28. 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.
29. Polovynko IS, Zajats LM, Popovych AI, Popovych IL. Integrated quantitative evaluation of neuro-endocrine and immune responses to chronic stress at rats male [in Ukrainian]. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 182.
30. Polovynko IS, Zayats' LM, Zukow W, Popovych IL. Neuro-endocrine-immune relationships by chronic stress at male rats. Journal of Health Sciences. 2013; 3(12): 365-374.
31. Polovynko IS, Zajats LM, Zukow W, Yanchij RI, Popovych IL. Quantitative evaluation of integrated neuro-endocrine and immune responses to chronic stress rats male. Journal of Education, Health and Sport. 2016; 6(8): 154-166.
32. Popovych IL (editor). General Adaptation Reactions and Body's Resistance in Liquidators of the Chernobyl Accident [in Ukrainian]. Kyiv: Computerpress. 2000. 117 p.
33. Попович ІЛ. Факторний і канонікальний аналізи параметрів нейро-ендокринно-імунного комплексу, метаболізму та ерозивно-виразкових пошкоджень слизової шлунку у щурів за умов гострого водно-імерсійного стресу. Медична гідрологія та реабілітація. 2007; 5(2): 68-80. Popovych IL. Factor and canonical analysis parameters of neuro-endocrine-immune complex, metabolism and erosive and ulcerative injuries of stomach mucosa in rats under acute water-immersion stress [in Ukrainian]. Medical Hydrology and Rehabilitation. 2007; 5(2): 68-80.
34. Popovych IL. Stresslimiting Adaptogene Mechanism of Biological and Curative Activity of Water Naftussya [in Ukrainian]. Kyiv: Computerpress. 2011. 300 p.
35. Popovych IL. The concept of neuro-endocrine-immune complex (review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18.
36. Popovych IL, Hrytsak YL, Barylyak LG, Popovych AI, Zukow W. Principal adaptation hormones blood levels correlated with parameters of immunity at healthy women and men. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 20.
37. Popovych IL, Kozyavkina OV. Immediate vegetotropic effects of bioactive water Naftussya and their neuro-endocrine-immune accompaniment in healthy men [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(3): 31-64.
38. Popovych IL, Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Barylyak LG, Tkachuk SP. Neuro-immune relationships in patients with chronic pyelonephritis and cholecystitis in conditions for balneotherapy on spa Truskavets'. Proceedings VIII Scientific Conference "Issues of pathology in conditions of extreme factors action on the body" (Ternopil', 1-2 October 2015). Ternopil'. 2015: 73-74.
39. Popovych IL, Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Barylyak LG, Zukow W. Neuro-immune relationships and homunculus conception. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 21.
40. 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.
41. Radchenko OM. Adaptation Reactions in Internal Diseases Clinic [in Ukrainian]. Lviv: League-Press. 2004. 232 p.
42. Schauenstein K, Felsner P, Rinner I, Liebmann PM, Stevenson JR, Westermann J, Haas HS, Cohen RL, Chambers DA. *In Vivo* Immunomodulation by Peripheral Adrenergic and Cholinergic Agonist/Antagonists in

Rat and Mouse Models. Neuroimmunomodulation. Perspectives at the New Millenium. Ann NYAS. 2000; 917: 618-627.
43. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific response to pathogens. Nat Rev Immunol. 2006; 6(4): 318-328.
44. Straub RH, Pongratz G, Weidler C, Linde H-J, Kirschning CJ, Glück T, Schölmerich J, Falk W. Ablation of the Sympathetic Nervous System Decreases Gram-Negative and Increases Gram-Positive Bacterial Dissemination: Key Roles for Tumor Necrosis Factor/Phagocytes and Interleukin-4/Lymphocytes. J Infect Dis. 2005; 192(4): 560-572.
45. Straub RH, Wiest R, Strauch UG, Härle P, Schölmerich J. The role of the sympathetic nervous system in intestinal inflammation. Gut. 2006; 55: 1640-1649.
46. Sydoruk NO, Zukow W. Comparative investigation of immediate effects on neuro-endocrine-immune complex of bioactive water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a. Communication 1. Generic effects. Journal of Education, Health and Sport. 2016; 6(8): 85-101.
47. Sydoruk NO, Zukow W, Yanchij RI. Integrated quantitative assessment of changes in neuro-endocrine-immune complex and metabolism in rats exposed to acute cold-immobilization stress. Journal of Education, Health and Sport. 2016; 6(9): 724-735.
48. Thayer JF, Fischer JE. Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. J Int Med. 2009; 265: 439-447.
49. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.
50. Tracey KJ. Understanding immunity requires more than immunology. Nature Immunology. 2010; 11(7): 561-564.
51. Uchakin PN, Uchakina ON, Tobin BV, Yershov FI. Neuroendocrine Immunomodulation [in Russian]. Vestnik Ross. AMN. 2007; 9: 26-32.
52. Вісьтак ГІ, Попович ІЛ. Вегетотропні ефекти біоактивної води Нафтуся та їх ендокринний і імунний супроводи у щурів-самок. Медична гідрологія та реабілітація. 2011; 9(2): 39-57. Vis'tak HI, Popovych IL. Vegetotropic effects of bioactive water Naftussya and their endocrine and immune support in female rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 39-57.

References in transliteration

1. Akmayev PG. Modern understanding of the interactions of regulatory systems: the nervous, endocrine and immune [in Russian]. Uspekhi fiziologicheskikh nauk. 1996; 27(1): 3-20.
2. 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.
3. Baevskiy RM, Ivanov GG. Heart Rate Variability: theoretical aspects and possibilities of clinical application [in Russian]. Ultrazvukovaya i funktsionalnaya diagnostika. 2001; 3: 106-127.
4. 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.
5. Chrousos GP. The stress response and immune function: clinical implications. The 1999 Novera H Spector lecture. Neuroimmunomodulation. Perspectives at the new millenium. ANYAS. 2000; 917: 38-67.
6. Czura SJ, Tracey KJ. Autonomic neural regulation of immunity. J Intern Med. 2005; 257(2): 156-166.
7. Denyssenko PP. Role of Cholinereactive Systems in Processes of Regulation [in Russian]. Moskwa: Meditsina. 1980. 296 p.
8. Dhabhar FS. Enhancing versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection and Immunopathology. Neuroimmunomodulation. 2009; 16(5): 300–317.
9. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil. 1981. 110 p.
10. Garkavi LKh, Kvakina YeB, Kuz'menko TS. Antistress Reactions and Activation Therapy [in Russian]. Moskva: Imedis, 1998. 654 p.
11. Garkavi LKh, Kvakina EB, Ukolova MA. Adaptation Reactions and Resistance of Organism [in Russian]. Rostov na Donu: Publishing House of Rostov University Press, 3rd ed. 1990. 224 p.

12. Hrytsak YaL, Barylyak LG, Zukow W, Popovych IL. Cluster analysis of hormonal constellation at women and men with harmonious and disharmonious general adaptation reactions. Journal of Education, Health and Sport. 2016; 6(4): 141-150.
13. Hrytsak YaL, Barylyak LG, Kovbasnyuk MM, Popovych IL. Plasma levels principal adaptation hormones and parameters of immunity closely interact. In: Valeology: current status, trends and persrectives of development. Abstracts. XIV Intern. scient. and practical conf. (Kharkiv-Drohobych, 14-16 April 2016). Kharkiv: VN Karazin KhNU. 2016: 354-356.
14. Khaitov RM. Physiology of the Immune System [in Russian]. Moskwa: VINITI RAS. 2005. 428 p.
15. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika. 1989: 78-138.
16. Kolyada TI, Volyans'kyi YuL, Vasilyev NV, Mal'tsev VI. Adaptation Syndrome and Immunity [in Russian]. Kharkiv: Osnova. 1995. 368 p.
17. Korneva EA (editor). Immunophysiology [in Russian]. St-Pb: Nauka. 1993. 684 p.
18. Kostyuk PG, Popovych IL, Ivassivka SV, Chebanenko LO, Kyjenko VM (editors). Chornobyl', Adaptive and Defensive Systems, Rehabilitation [in Ukrainian]. Kyiv: Computerpress. 2006. 348 p.
19. Kozyavkina OV. Vegetotropic effects of bioactive water Naftyssya at children with dysfunction of the neuroendocrine-immune complex, these endocrine-immune accompaniment and the ability to predict [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 24-39.
20. Kozyavkina OV. Vegetotropic effects of bioactive water Naftyssya at males rats and their endocrine, electrolyte iand mmune accompaniments [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(3): 65-92.
21. 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.
22. Kozyavkina OV, Popovych IL, Zukow W. Immediate vegetotropic effects of bioactive water Naftussya and their neuro-endocrine-immune accompaniment in healthy men. Journal of Health Sciences. 2013; 3(5): 391-408.
23. Kul'chyns'kyi AB. The relationships between changes in parameters of Electroencephalogram and Immunogram in the conditions balneotherapy on spa Truskavets' [in Ukrainian]. In: Valeology: current status, trends and persrectives of development. Abstracts. XIV Intern. scient. and practical. conf. (Kharkiv-Drohobych, 14-16 April 2016). Kharkiv: VN Karazin KhNU. 2016: 322-323.
24. Lapovets' LYe, Lutsyk BD. Handbook of Laboratory Immunology [in Ukrainian]. L'viv. 2002. 173 p.
25. Mandel ID. Cluster analysis [trans. from English in Russian]. Moskva: Finansy i Statistika. 1988. 176 p.
26. Nance DM, Sanders VM. Autonomic innervation and regulation of immune system (1987-2007). Brain Behav Immun. 2007; 21(6): 736-745.
27. Petsyukh SV, Petsyukh MS. Leukocytary Popovych's adaptation index correlated with parameters of ongoiging HRV and EEG. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 19.
28. 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.
29. Polovynko IS, Zajats LM, Popovych AI, Popovych IL. Integrated quantitative evaluation of neuro-endocrine and immune responses to chronic stress at rats male [in Ukrainian]. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 182.
30. Polovynko IS, Zayats' LM, Zukow W, Popovych IL. Neuro-endocrine-immune relationships by chronic stress at male rats. Journal of Health Sciences. 2013; 3(12): 365-374.
31. Polovynko IS, Zajats LM, Zukow W, Yanchij RI, Popovych IL. Quantitative evaluation of integrated neuro-endocrine and immune responses to chronic stress rats male. Journal of Education, Health and Sport. 2016; 6(8): 154-166.
32. Popovych IL (editor). General Adaptation Reactions and Body's Resistance in Liquidators of the Chernobyl Accident [in Ukrainian]. Kyiv: Computerpress. 2000. 117 p.
33. Popovych IL. Faktornyj i kanonikal"nyj analizy parametriv nejro-endokrynno-imunnoho kompleksu, metabolizmu ta erozyvno-vyrazkovyx poshkodzhen" slyzovoyi shlunku u shhuriv za umov hostroho vodno-imersijnoho stresu. Medychna hidrolohiya ta reabilitaciya. 2007; 5(2): 68-80. Popovych IL. Factor and canonical analysis parameters of neuro-endocrine-immune complex, metabolism and erosive and ulcerative injuries of stomach mucosa in rats under acute water-immersion stress [in Ukrainian]. Medical Hydrology and Rehabilitation. 2007; 5(2): 68-80.

34. Popovych IL. Stresslimiting Adaptogene Mechanism of Biological and Curative Activity of Water Naftussya [in Ukrainian]. Kyiv: Computerpress. 2011. 300 p.
35. Popovych IL. The concept of neuro-endocrine-immune complex (review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18.
36. Popovych IL, Hrytsak YL, Barylyak LG, Popovych AI, Zukow W. Principal adaptation hormones blood levels correlated with parameters of immunity at healthy women and men. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 20.
37. Popovych IL, Kozyavkina OV. Immediate vegetotropic effects of bioactive water Naftussya and their neuro-endocrine-immune accompaniment in healthy men [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(3): 31-64.
38. Popovych IL, Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Barylyak LG, Tkachuk SP. Neuro-immune relationships in patients with chronic pyelonephritis and cholecystitis in conditions for balneotherapy on spa Truskavets'. Proceedings VIII Scientific Conference "Issues of pathology in conditions of extreme factors action on the body" (Ternopil', 1-2 October 2015). Ternopil'. 2015: 73-74.
39. Popovych IL, Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Barylyak LG, Zukow W. Neuro-immune relationships and homunculus conception. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv: Publishing National Pharmaceutic University. 2016: 21.
40. 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.
41. Radchenko OM. Adaptation Reactions in Internal Diseases Clinic [in Ukrainian]. Lviv: League-Press. 2004. 232 p.
42. Schauenstein K, Felsner P, Rinner I, Liebmann PM, Stevenson JR, Westermann J, Haas HS, Cohen RL, Chambers DA. In Vivo Immunomodulation by Peripheral Adrenergic and Cholinergic Agonist/Antagonists in Rat and Mouse Models. Neuroimmunomodulation. Perspectives at the New Millenium. Ann NYAS. 2000; 917: 618-627.
43. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific response to pathogens. Nat Rev Immunol. 2006; 6(4): 318-328.
44. Straub RH, Pongratz G, Weidler C, Linde H-J, Kirschning CJ, Glück T, Schölmerich J, Falk W. Ablation of the Sympathetic Nervous System Decreases Gram-Negative and Increases Gram-Positive Bacterial Dissemination: Key Roles for Tumor Necrosis Factor/Phagocytes and Interleukin-4/Lymphocytes. J Infect Dis. 2005; 192(4): 560-572.
45. Straub RH, Wiest R, Strauch UG, Härle P, Schölmerich J. The role of the sympathetic nervous system in intestinal inflammation. Gut. 2006; 55: 1640-1649.
46. Sydoruk NO, Zukow W. Comparative investigation of immediate effects on neuro-endocrine-immune complex of bioactive water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a. Communication 1. Generic effects. Journal of Education, Health and Sport. 2016; 6(8): 85-101.
47. Sydoruk NO, Zukow W, Yanchij RI. Integrated quantitative assessment of changes in neuro-endocrine-immune complex and metabolism in rats exposed to acute cold-immobilization stress. Journal of Education, Health and Sport. 2016; 6(9): 724-735.
48. Thayer JF, Fischer JE. Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. J Int Med. 2009; 265: 439-447.
49. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.
50. Tracey KJ. Understanding immunity requires more than immunology. Nature Immunology. 2010; 11(7): 561-564.
51. Uchakin PN, Uchakina ON, Tobin BV, Yershov FI. Neuroendocrine Immunomodulation [in Russian]. Vestnik Ross. AMN. 2007; 9: 26-32.
52. Vis"tak HI, Popovych IL. Vehetotropni efekty bioaktyvnoyi vody Naftusya ta yix endokrynnyj i imunnyj suprovody u shhuriv-samok. Medychna hidrolohiya ta reabilitaciya. 2011; 9(2): 39-57. Vis'tak HI, Popovych IL. Vegetotropic effects of bioactive water Naftussya and their endocrine and immune support in female rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 39-57.