Lukyanchenko Oleksandr I, Gozhenko Olena A, Mel'nyk Oksana I, Zukow Walery, Popovych Igor L. Features of the immune profile and microbiota in persons whose immune status is susceptible or resistant to chronic stress. Journal of Education, Health and Sport. 2019;9(3):601-611. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.2648087
http://ojs.ukw.edu.pl/index.php/johs/article/view/6845

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: 01.03.2019. Revised: 15.03.2019. Accepted: 26.03.2019.

FEATURES OF THE IMMUNE PROFILE AND MICROBIOTA IN PERSONS WHOSE IMMUNE STATUS IS SUSCEPTIBLE OR RESISTANT TO CHRONIC STRESS

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Abstract

Background. The presence of influence both sympathetic and vagus links of the autonomic nervous system to the immune system is generally recognized, but the data on their specific immunotropic effects are ambiguous. This includes, in particular, immune responses to the stress-induced shift of sympatho-vagal balance. The purpose of this study is to identify the characteristics of the immune profile, as well as the microbiota associated with it, in persons whose immune status is susceptible or resistant to chronic stress. **Materials and Methods.** The object of observation were 32 men and 8 women with chronic pyelonephritis in remission. The criterion for inclusion was the magnitude of the sympatho-vagal balance index LF/HF (recorded by "CardioLab+HRV"), which exceeded the age norm by 0,5 σ. Immune status evaluated on a set of I and II levels recommended by the WHO. The observed contingent by pair matching of persons with the same values of LF/HF ratio was retrospectively divided into two groups, almost identical to the average value of LF/HF and its dispersion, but with opposite deviations from the norm of the immune status index. **Results.** Immune profiles constructed on Z-scores can be divided into three networks. The first set contains 8 parameters (Killing Index vs E. coli and Staph. aur., IL-6, Entropy of LCG, Popovych's Strain Index of LCG, Igg G and M as well as Microbial Count E. coli) that are not significantly different from persons who are stress-sensitive and stress-resistant. 18 parameters of the second set (Leukocytes, Phagocytose Index vs E. coli and Staph. aur.

(PhIA), B-Lymphocytes, Segmented Neutrophils, Microbial Count Staph. aur., T-active (Ta), T-cytolytic and Natural Killers Lymphocytes (NK), IgA, Bactericidity vs E. coli and Staph. aur. (BCA), Bifidobacterium and Lactobacillus feces, Stub Neutrophils, T-helpers, Popovych's Adaptation Index of LCG (PAI) as well as CIC) to a greater or lesser degree higher in stress-resistant persons. Instead, the 8 parameters of the third set (Lymphocytes, Monocytes and Eosinophils (E), TNF-α, IL-1 as well as Popovych's Strain Index of LCG, 0-Lymphocytes and Hemolytica E. coli HEC) are higher in stress-sensitive persons. The method of discriminant analysis revealed 9 parameters (ranked by criterion Λ: 0-Lym, BCA, HEC, Ta, CIC, E, PhIA, PAI, NK) that characterize the features of immune profile of stress-susceptible and stress-resistant persons.

Keywords: immune profile; microbiota; chronic stress; stress-susceptible and stress-resistant persons.

INTRODUCTION

The presence of influence both sympathetic and vagus links of the autonomic nervous system to the immune system is generally recognized, but the data on their specific immunotropic effects are ambiguous. This includes, in particular, immune responses to the stress-induced shift of sympatho-vagal balance [4,6,7,10,12,13,19,22-24,27] as well as to vegetotropic factors [26]. Consequently, research in this area remains relevant. Previously, we have shown that on the day after four hours of water immersing cold stress, the state of the gastic mucosa of rats as a classical target of stress realising systems shows a variation from the lack of visible changes to multiple ulcers [15], that is, there is a basis for the assertion of its individual sensitivity to stress. The method of discriminant analysis revealed a number of immune parameters (spleen mass, content in splenocytogram of reticulocytes and macrophages, content in thymocytogram of lymphocytes, basophils, fibroblastes, macrophages and Hassal's corpusculs, intensity and completeness of phagocytosis of Staph. aureus by blood neutrophils, blood levels of cytolytic and helper T-lymphocytes as well as natural killers), the totality of which groups of rats without seen damages, erosions, ulcerations, including it is not enough, middle and badly expressed, differ significantly from each other as well as from the intact group [16]. That is, there is a basis for the assertion of individual sensitivity of immunity to stress. Such a position was confirmed in the monitoring of children who were in a state of chronic stress. By pair comparison of individuals with the same values of Baevskiy's stress index in one group of children the bactericidal ability of neutrophils was significantly reduced, while the other was close to normal [25].

The purpose of this study is to identify the characteristics of the immune profile, as well as the microbiota associated with it, in persons whose immune status is susceptible or resistant to chronic stress.

MATERIAL AND METHODS

The object of observation were 32 men (aged 24-70 years old) and 8 women (39-71 ys) with chronic pyelonephritis in remission. The criterion for inclusion was the magnitude of the sympatho-vagal balance index LF/HF, which exceeded the age norm by 0,5 σ.

To this end, we recorded electrocardiogram in II lead to assess the parameters of HRV [1,3] (software and hardware complex "CardioLab+HRV" production "KhAI-MEDICA", Kharkiv, Ukraine). For further analysis the following parameters heart rate variability (HRV) were selected. Temporal parameters (Time Domain Methods): the standard deviation of all NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater than 50 ms (pNN_{50}). Spectral parameters (Frequency Domain Methods): spectral power density (SPD) bands of HRV: high-frequency (HF, range 0,4÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,04÷0,015 Hz) and ultra low-frequency (ULF, range 0,015÷0,003 Hz). We calculated classical indexes: LF/HF, LFnu=100%•LF/(LF+HF) and Baevskiy's Activity Regulatory Systems Index (BARSI) [1] as well as the Entropy (h) of normalized SPD using CE Shannon's formula:

$$hHRV = -[SPD\ HF \bullet \log_2 SPD\ HF + SPD\ LF \bullet \log_2 SPD\ LF + SPD\ VLF \bullet \log_2 SPD\ VLF + SPD\ ULF \bullet \log_2 SPD\ ULF]/\log_2 4$$

Immune status evaluated on a set of I and II levels recommended by the WHO [14]. 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). Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [5] with moderately modification by MM Kovbasnyuk [11]. 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". Take into account the following parameters of phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index), intensity (number of microbes absorbed one phagocytes - Microbial Count or Right's Index) and completeness (percentage of dead microbes - Killing Index). Most interesting is the integrated evaluation of phagocytic function of neutrophils by the number of microbes that are able to neutralize by neutrophils contained in 1 L of blood, named as Bactericidity Capacity (BCC) and calculated by formula [9,21]:

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

We counted up also Leukocytogram and calculated its Entropy by CE Shannon as well as Adaptation Index and Strain Index by IL Popovych [2,9,18]. In addition, the blood level of cytokines IL-1, IL-6 and TNF-α was determined (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Diactone", France). The condition of microbiota is evaluated on the results of sowing of feces and urine.

Results processed using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Preliminary results are published in the abstract format [17].

For the purpose of a one-scale evaluation of various parameters, all of them were listed in the Z-score by the formula [6,27]: Z = (Variable/Norm -1)/Coefficient of Variation.

LF/HF ratio as the criterion for inclusion was in the range of 2,2 to 23,8 units or of 0,51 to 17,65 Z-score indicating the state of stress.

Integral state of Phagocytosis (Phag-2), assessed by BCC against Staph. aureus and E. coli, was in the range of -4,85 to +6,24. Integral state of Cellular link (Cel-4), assessed by levels of $CD3^+$ T-active Lymphocytes, $CD3^+CD4^+$ T-helpers, $CD3^+CD8^+$ T-killers and $CD16^+$ Natural killers, was in the range of -3,24 to +2,49. Integral state of Humoral link (Hum-5), assessed by levels of $CD22^+$ B-Lymphocytes, M, G and A Immunoglobulins as well as Circulating Immune Complexes, was in the range of -1,36 to +1,57. The level of immunity in general, evaluated by the algebraic sum of states of its three links (ID-11), was in the range of -2,53 to +1,34.

The observed contingent by pair matching of persons with the same values of LF/HF ratio was retrospectively divided into two groups, almost identical to the average value of LF/HF and its dispersion, but with opposite deviations from the norm of the immune status index (ID-11).

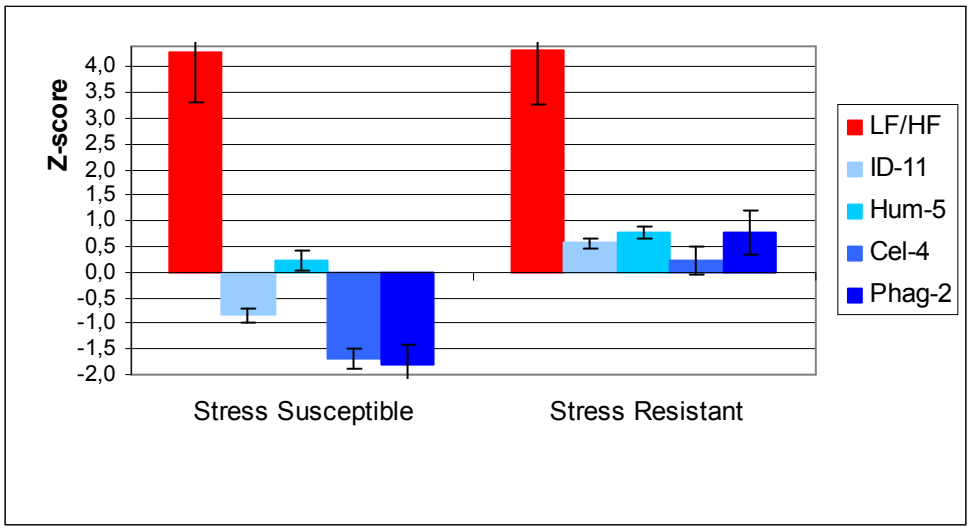

Fig. 1. Condition of immunity in general and its humoral, cellular and phagocytic links in persons stress-sensitive and stress-resistant

A comparative analysis of parameters of each links of immune system is given in the tables 1-4.

Table 1. Parameters of Leukocytogram in persons who are stress-sensitive and stress-resistant

Variables	Norm (30)	Cv	SS (20)	SR (20)	p SS/N	p SR/N	p SR/SS
Leukocytes total, 10^9/L	5,00±0,09	0,100	5,51±0,24	6,17±0,27	>0,05	<0,001	>0,05
Segmented Neutrophils, %	55,0±1,0	0,100	53,5±1,9	58,8±1,0	>0,5	=0,01	<0,05
Stub Neutrophils, %	4,25±0,11	0,147	2,4±0,3	3,5±0,2	<0,001	<0,01	<0,01
Lymphocytes, %	32,0±1,0	0,174	33,0±1,7	28,5±0,7	-	<0,01	<0,02
Monocytes, %	6,0±0,2	0,167	7,2±0,9	6,2±0,8	>0,2	-	>0,5
Eosinophils, %	2,75±0,16	0,318	3,9±0,6	2,9±0,3	>0,05	>0,5	>0,1

Table 2. Parameters of Phagocytosis in persons who are stress-sensitive and stress-resistant

Variables	Norm (30)	Cv	SS (20)	SR (20)	p SS/N	p SR/N	p SR/SS
Neutrophils, 10^9/L	2,96±0,05	0,100	3,03±0,11	3,82±0,14	-	<0,001	<0,001
Phagocytose Index vs Staph. aur, %	98,3±0,3	0,018	98,6±0,3	99,1±0,2	-	<0,05	>0,2
Microbial Count Staph. aur, B/Ph	61,6±0,9	0,080	60,1±1,9	64,0±1,6	-	>0,2	>0,2
Killing Index vs Staph. aur, %	58,9±0,8	0,071	47,7±1,8	46,2±1,4	<0,001	<0,001	>0,5
Bactericidity vs St. aur, 10^9 Bac/L	106±2	0,100	85±4	111±6	<0,001	>0,2	<0,001
Phagocytose Index vs E. coli, %	98,3±0,2	0,012	98,7±0,4	99,6±0,1	-	<0,001	<0,05
Microbial Count E. coli, Bac/Phag	54,7±1,0	0,097	64,8±1,8	65,3±1,7	<0,001	<0,001	-
Killing Index vs E. coli,%	62,0±0,9	0,078	43,6±2,7	44,8±1,9	<0,001	<0,001	-
Bactericidity vs E. coli, 10^9 Bac/L	99±2	0,100	82±5	109±4	<0,01	<0,05	<0,001
Phagocytose Link-2, Z	0		-1,82±0,39	+0,76±0,43	<0,001	>0,05	<0,001

Table 3. Parameters of Cellular Immunity in persons who are stress-sensitive and stress-resistant

Variables	Norm (30)	Cv	SS (20)	SR (20)	p SS/N	p SR/N	p SR/SS
$CD3^+$ T-active Lymphocytes, %	30,0±0,9	0,167	26,8±1,0	32,0±1,1	<0,05	>0,1	<0,001
$CD3^+$ $CD4^+$ T-helpers, %	39,5±0,6	0,082	27,3±1,4	36,2±1,7	<0,001	>0,05	<0,001
$CD3^+$ $CD8^+$ T-cytolytic, %	23,5±0,6	0,138	21,0±1,1	25,2±0,8	>0,05	<0,02	<0,01
$CD16^+$ Natural Killers, %	17,0±0,5	0,172	12,3±0,9	20,0±1,2	<0,001	<0,05	<0,001
Cellular Immunity Link-4, Z	0		-1,69±0,21	+0,23±0,26	<0,001	>0,2	<0,001

Table 4. Parameters of Humoral Immunity in persons who are stress-sensitive and stress-resistant

Variables	Norm (30)	Cv	SS (20)	SR (20)	p SS/N	p SR/N	p SR/SS
$CD22^+$ B-Lymphocytes, %	20,0±0,6	0,175	23,0±1,2	25,4±1,0	<0,05	<0,001	>0,1
IgG, g/L	12,8±0,5	0,206	14,9±1,1	15,2±0,6	>0,1	<0,01	-
IgM, g/L	1,15±0,05	0,239	1,40±0,07	1,46±0,06	<0,01	<0,001	>0,5
IgA, g/L	1,87±0,06	0,167	1,68±0,12	2,02±0,07	>0,1	>0,1	<0,02
CIC, units	45,0±3,2	0,389	30,1±2,8	40,3±3,7	<0,01	>0,5	<0,05
Humoral Immunity Link-5, Z	0		+0,23±0,19	+0,76±0,11	>0,2	<0,001	<0,02

Given the link between the parameters of immunity and microbiota intestine and urine [9,12,21], the latter also became the object of comparative analysis (Table 5).

Table 5. Parameters of microbiota feces and urine in persons who are stress-sensitive and stress-resistant

Variables	Norm (30)	Cv	SS (20)	SR (20)	p SS/N	p SR/N	p SR/SS
Lactobacillus, lg CFU/g	8,10±0,12	0,015	5,53±0,41	6,40±0,23	<0,001	<0,001	>0,05
Bifidobacterium, lg CFU/g	6,94±0,08	0,011	5,04±0,33	5,68±0,19	<0,001	<0,001	>0,1
Escherichia coli, lg CFU/g	8,30±0,02	0,013	8,18±0,10	8,21±0,05	>0,2	>0,1	>0,5
Attenuated E. coli, %	17±1	0,309	65±7	67±5	<0,001	<0,001	-
Hemolytica E. coli, %	0		35±10	12±7	<0,01	>0,1	>0,05
Klebsiela&Proteus, %	10±1	0,5	18±5	13±2	>0,1	>0,2	>0,2
Bacteriuria, lg CFU/L	-0,60±0,04	0,360	1,79±0,19	0,62±0,15	<0,001	<0,001	<0,001
Bacteriuria, points	0		0,40±0,05	0,12±0,03	<0,001	<0,001	<0,001
Leukocyturia, lgL/mL	3,00±0,04	0,070	3,42±0,17	3,61±0,12	=0,02	<0,001	>0,2

Since the source of the cytokines is all the populations of leukocytes [7], they are collected in a separate table 6. It also includes 0-lymphocytes, the content of which is calculated by the balance method, as well as information parameters: Entropy of Immunocytogram (ICG) and Leukocytogram (LCG) and Leukocytary Strain and Adaptation Indexes [2,18].

Table 6. Parameters of calculated and non-system immunity parameters in persons who are stress-sensitive and stress-resistant

Variables	Norm (30)	Cv	SS (20)	SR (20)	p SS/N	p SR/N	p SR/SS
0-Lymphocytes, %	0		16,4±2,6	-6,8±3,1	<0,001		<0,001
Entropy of ICG	0,96±0,01	0,059	0,90±0,01	0,98±0,01	<0,001	>0,1	<0,001
Entropy of LCG	0,68±0,01	0,073	0,67±0,01	0,65±0,01	>0,5	<0,02	>0,2
Strain Index of LCG	0,06±0,01	0,618	0,46±0,15	0,20±0,08	<0,02	>0,05	>0,1
Adaptation Index of LCG	1,70±0,05	0,147	0,87±0,10	1,10±0,10	<0,001	<0,001	>0,05
IL-6, ng/L	4,25±0,25	0,324	1,15±0,12	0,90±0,11	<0,001	<0,001	>0,1
TNF-α, ng/L	4,90±0,29	0,326	5,67±0,76	3,93±0,60	>0,2	>0,1	>0,05
IL-1, ng/L	4,51±0,14	0,173	5,22±0,25	4,46±0,25	<0,05	-	<0,05

Immune profiles constructed on Z-scores can be divided into three networks (Figure 2). The first set contains 8 parameters (**Killing Index vs E. coli and Staph. aur., IL-6, Entropy of LCG, Popovych's Strain Index of LCG, Igg G and M** as well as **Microbial Count E. coli**) that are not significantly different from persons who are stress-sensitive and stress-resistant. 18 parameters of the second set (**Leukocytes, Phagocytose Index vs E. coli and Staph. aur., B-Lymphocytes, Segmented Neutrophils, Microbial Count Staph. aur., T-active, T-cytolytic and Natural Killers Lymphocytes, IgA**, **Bactericidity vs E. coli and Staph. aur., Bifidobacterium and Lactobacillus feces, Stub Neutrophils, T-helpers, Popovych's Adaptation Index of LCG** as well as **CIC**) to a greater or lesser degree higher in stress-resistant persons. Instead, the 8 parameters of the third set (**Lymphocytes**, **Monocytes and Eosinophils, TNF-α, IL-1** as well as not shown in the figure **Strain Index of LCG, 0-Lymphocytes** and **Hemolytica E. coli feces**) are higher in stress-sensitive persons.

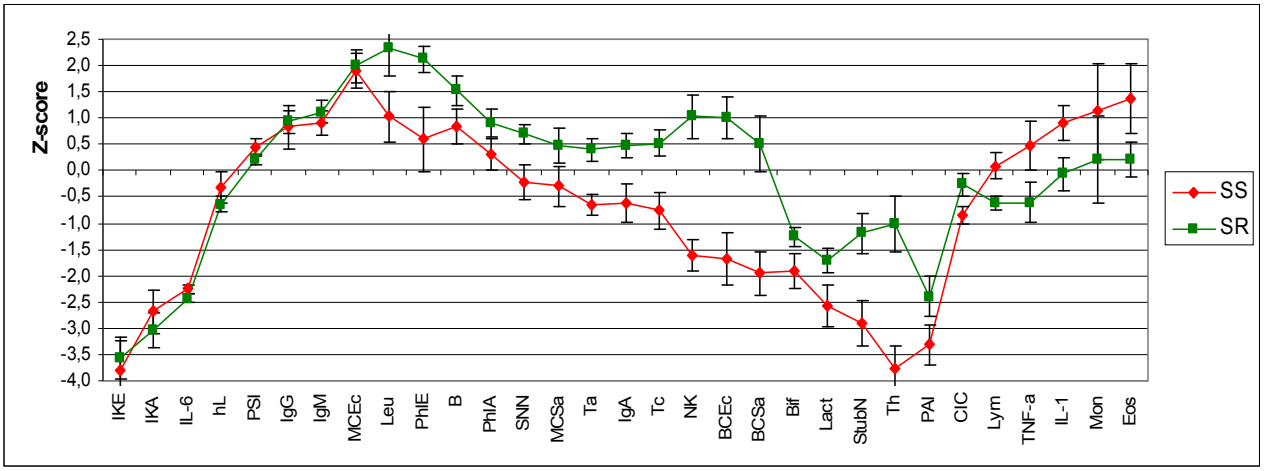

Fig. 2. Immune profiles of persons sensitive (SS) and resistant (SR) to stress

The method of discriminant analysis forward stepwise [8] revealed 9 parameters (variables) that characterize the features of immune profile of stress-sensitive and stress-resistant persons (Tables 7 and 8).

Table 7. Summary of Stepwise Analysis. The scale of ranks for variables

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
0-Lymphocytes, %	32,1	10^{-5}	,542	32	10^{-5}
Bactericidity vs St. aur, 10^9 B/L	22,9	10^{-4}	,335	37	10^{-6}
Hemolytica E. coli feces, %	3,4	,074	,306	27	10^{-6}
CD3$^+$ T-active Lymphocytes, %	4,9	,034	,269	24	10^{-6}
Circulating Immune Complex, Z	2,8	,103	,248	21	10^{-6}
Eosinophils, %	2,8	,104	,229	19	10^{-6}
Phagocytose Index vs St. aur, %	2,6	,115	,211	17	10^{-6}
Adaptation Index of LCG	1,2	,288	,204	15	10^{-6}
CD16$^+$ Natural Killers, %	1,0	,323	,197	14	10^{-6}

Table 8. Discriminant Function Analysis Summary for parameters of Immunity and Microbiota

Step 9, N of vars in model: 9; Grouping: SS&SR

Wilks' Lambda: 0,1971; approx. $F_{(9,3)}$=13,6; $p<10^{-6}$

Variables currently in the model	**SS (20)**	**SR (20)**	Wilks' Λ	Partial Λ	F remove	p-level	Tolerancy
0-Lymphocytes, %	16,4±2,6	-6,8±3,1	,205	,961	1,2	,279	,160
Bactericidity vs St. aur, 10^9 B/L	85±4	111±6	,360	,547	24,8	10^{-4}	,640
Hemolytica E. coli feces, %	35±10	12±7	,249	,791	7,9	,008	,562
CD3$^+$ T-active Lymphocytes, %	26,8±1,0	32,0±1,1	,250	,789	8,0	,008	,599
Circulating Immune Complex, Z	30,1±2,8	40,3±3,7	,235	,838	5,8	,022	,677
Eosinophils, %	3,9±0,6	2,9±0,3	,204	,968	1,0	,328	,530
Phagocytose Index vs St. aur, %	98,6±0,3	99,1±0,2	,212	,928	2,3	,138	,688
Adaptation Index of LCG	0,87±0,10	1,10±0,10	,209	,944	1,8	,191	,649
CD16$^+$ Natural Killers, %	12,3±0,9	20,0±1,2	,204	,968	1,0	,324	,172

Information about the variables included in the model is condensed in the canonical discriminant root. The sum of products of raw coefficients on the value of discriminant variables together with the constant gives the values of roots for each person and allow their visualization (Fig. 3).

Table 9. Z-scores of Discriminant Variables and Standardized, Structural and Raw Coefficients and Constant for them

Variables currently in the model	**Stress-snsceptible**	**Stress-resistant**	Standar-dized	Structural	Raw
$CD16^+$ Natural Killers, Z	-1,62±0,30	+1,03±0,41	,485	**,422**	,104
Bactericidity vs Staph. aureus, Z	-1,96±0,42	+0,51±0,53	,938	**,293**	,041
$CD3^+$ T-active Lymphocytes, Z	-0,64±0,20	+0,40±0,21	,662	**,286**	,143
Circulating Immune Complex, Z	-0,85±0,16	-0,27±0,21	,547	**,176**	,037
Adaptation Index of LCG, Z	-3,31±0,39	-2,40±0,38	-,329	**,136**	-,768
Phagocytose Index vs St. aur, Z	+0,32±0,29	+0,90±0,27	-,361	**,117**	-,322
Eosinophils, Z	+1,37±0,66	+0,22±0,34	,274	-,125	,133
0-Lymphocytes, %	16,4±2,6	-6,8±3,1	-,549	-,455	-,042
Hemolytica E. coli feces, %	35±10	12±7	-,682	-,155	-,018
	Eigenvalue=4,073			**Constant**	21,51
r^*=0,896; Wilks' Λ=0,197; $\chi^2_{(9)}$=54; $p=10^{-6}$					
Squared Mahalanobis Distance=16,3; F=13,6; $p=10^{-6}$					

Note. The last two variables are expressed in actual units because of the mathematical impossibility of computing the Z-score (Norm=0)

As you can see, **stress-susceptible** and **stress-resistant** persons are very clearly delineated: the maximum size of the discriminant canonical root of the first cluster is less than the minimum value of the second cluster. In this case, in **stress-susceptible** individuals below the norm, by definition, the level of $CD16^+$ Natural Killers, Bactericidity vs Staph. aureus, $CD3^+$ T-active Lymphocytes and CIC are accompanied by a reduced index of Adaptation, but a normal Phagocytosis index vs Staph. aureus and higher than normal levels of Eosinophils and 0-Lymphocytes, as well as Hemolytica E. coli feces. Instead, in the **stress-resistant** persons, the five parameters are within the norm, the lowering level of the Adaptation index is less tangible, whereas levels of Natural Killers and Phagocytosis index even exceed the norm.

The negative value of 0-Lymphocytes level deserves a special comment. Since their content in the immunocytogram is calculated by the formula: 0-L=100-Th-Tc-B-NK, the negative value indicates the presence of so-called D-Lymphocytes, that is, those expressing receptors, characteristic of both NK and B-Lymphocytes.

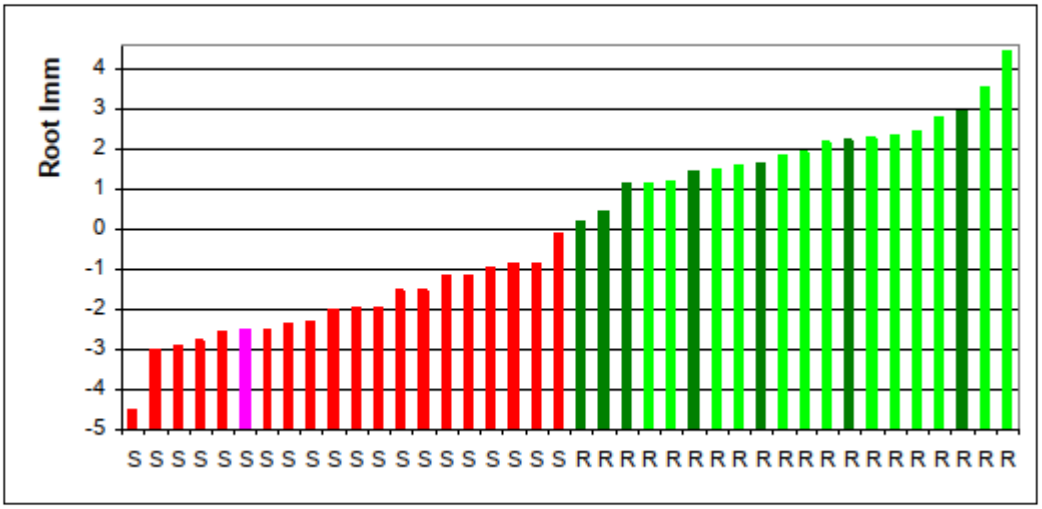

Fig. 3. Individual sizes of the immune discriminant root of persons sensitive (S) and resistant (R) to stress

Another aspect relates to the role of sex in the immune response to chronic stress. In each set women are identified with different colours. As you can see, both sexes are mixed in a stress-resistant set. In the stress-sensitive set, there was only one **woman**, but almost in the middle. Consequently, sex does not determine the nature of the immune response to chronic stress.

The role of the state of the nervous and endocrine systems as well as metabolism will be the subject of the next sex, which has already been prepared for printing.

In the end, we give Coefficients and Constants for Classification Functions (Table 10) for retrospective identification of stress-susceptible and stress-resistant persons.

Table 10. Coefficients and Constants for Classification Functions

Variables currently in the model	SS	SR
0-Lymphocytes, %	4,947	4,780
Bactericidity vs Staph. aureus, 10^9 Bacter/L	,058	,221
Hemolytica E. coli feces, %	,729	,657
$CD3^+$ T-active Lymphocytes, %	-4,051	-3,487
Circulating Immune Complexes, units	-1,549	-1,402
Eosinophils, %	-6,617	-6,094
Phagocytose Index vs Staph. aureus, %	111,7	110,4
Adaptation Index of Leukocytogram	-15,42	-18,44
$CD16^+$ Natural Killers, %	1,759	2,168
Constants	-5475	-5390

ACKNOWLEDGMENT

We express sincere gratitude to administration JSC “Truskavets’kurort” and “Truskavets’ SPA” as well as clinical sanatorium “Moldova” for help in conducting this investigation.

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.

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