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VEGETATIVE, ENDOCRINE AND METABOLIC ACCOMPANIMENTS OF INDIVIDUAL IMMUNE RESPONSES TO ADAPTOGENIC BALNEOTHERAPY

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Abstract
Background. Earlier in clinical observations four variants of the immune responses to adaptogenic balneotherapy at the Truskavets' spa have been identified. In 40,9% of patients, initially normal immune status did not change significantly; in 31,8%, the lower boundary level of immunity is completely normalized; in 22,7% moderate immunosuppression is reduced, but not up to normal; however, in 4,5% of people, initially normal level of immunity are transformed into moderate immunosuppression. All four variants of immune responses are virtually unmistakably predicted by a set of 20 predictors. The **purpose** of this study is to analyze vegetative, endocrine and metabolic accompaniments of individual immune responses to balneofactors of Truskavets' spa. **Material and methods.** The object of observation were 34 men and 10 women aged 24-70 years old, who came to the Truskavets' spa for the treatment of chronic pyelonephritis combined with cholecystitis in remission. The survey was conducted twice, before and after balneotherapy (drinking bioactive water Naftussya three times a day, ozokerite applications, mineral baths every other day for 7-10 days). Immune status evaluated on a set of I and II levels recommended by the WHO. The state of the autonomic nervous system is estimated by HRV ("CardioLab+HRV"). To assess endocrine status we determined plasma levels of principal adaptation Hormones: Cortisol, Testosterone and Triiodothyronine (ELISA). We determined also the plasma and daily urine levels of the electrolytes: calcium, phosphates, sodium, potassium, magnesium and chloride as well as nitrous metabolites creatinine, urea and uric acid. According to the parameters of electrolyte exchange, parathyroid, calcitonin and mineralocorticoid hormonal activities was evaluated. We estimated also plasma lipide spectrum. **Results.** 13 immunosuppressive and 6 immune-enhancing neuro-endocrine and metabolic parameters identified. Discriminant analysis conducted to identify parameters, in which the four immune response clusters differ significantly from each other. 25 parameters are characteristic, 12 of them related to the HRV, 5 to endocrine, 5 to electrolytes and 3 to nitrous metabolites exchange. **Conclusion**.

Individual immune responses to adaptogenic balneofactors are accompanied by characteristic changes in the parameters of the autonomic nervous and endocrine systems, as well as the exchange of electrolytes, nitrogen metabolites and lipids.

Key words: Individual immune responses; HRV, hormones, electrolytes, nitrogen metabolites, lipids, balneotherapy, Truskavet's spa.

INTRODUCTION

Earlier in clinical observations four variants of the immune responses to adaptogenic balneotherapy at the Truskavets' spa have been identified. In 40,9% of patients, initially normal immune status did not change significantly. In 31,8%, the lower boundary level of immunity is completely normalized. In 22,7% moderate immunosuppression is reduced, but not up to normal. However, in 4,5% of people, initially normal level of immunity are transformed into moderate immunosuppression [31,32]. All four variants of immune responses are virtually unmistakably predicted by a set of 20 predictors including 13 immune, 4 information, 2 fecal microbiota parameters as well as erythrocyturia [20].

The relationships between the immune, nervous and endocrine systems are well documented [3,4,21,25,29,34,35], in particular in condition of adaptogenic balneotherapy [12,15,17,18,23,24,26,28,30,33]. Also known about the links between the parameters of immunity and metabolism, in particular electrolytes, lipids, nitrogen metabolites [7-9,15].

Therefore, the **purpose** of this study is to analyze vegetative, endocrine and metabolic accompaniments of individual immune responses to balneofactors of Truskavets' spa as natural adaptogens.

MATERIAL AND METHODS

The object of observation were 34 men and 10 women aged 24-70 years old, who came to the Truskavets' spa for the treatment of chronic pyelonephritis combined with cholecystitis in remission. The survey was conducted twice, before and after balneotherapy (drinking bioactive water Naftussya three times a day, ozokerite applications, mineral baths every other day for 7-10 days [15,23,27]).

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manuals [13,19]. 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 CD56 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 Circulating Immune Complexes (by polyethylene glycol precipitation method) and Immunoglobulins classes M, G, A as well as in saliva of IgG, IgA, secretory IgA and Lysozyme (ELISA, analyser "Immunochem", USA).

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [5] with moderately modification by MM Kovbasnyuk [17,18,28]. 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 [15]:

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

The state of the autonomic nervous system is estimated by parameters of heart rate variability (HRV). We recorded electrocardiogram in II lead (software and hardware complex "CardioLab+HRV" production "KhAI-MEDICA", Kharkiv). For further analysis the following parameters HRV were selected [1,2,10]. Temporal parameters (Time Domain Methods): the standart 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 then 50 ms (pNN_{50}); heart rate (HR), the Mode (Mo), the Amplitude of Mode (AMo), variational sweep (MxDMn) as well as Triangulary Index (TINN). Spectral parameters (Frequency Domain Methods): spectral power (SP) 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). On the basis of these parameters were calculated proportion of SP bands (% of Total Power) and classical indexes: LF/HF, LFnu=100%•LF/(LF+HF), Centralization Index=(VLF+LF)/HF; Baevskiy's Stress Index (BSI=AMo/2•Mo•MxDMn) and Baevskiy's Activity Regulatory Systems Index (BARSI) [1] as well as the Entropy (h) of HRV [22].

To assess endocrine status in the morning on an empty stomach we determined plasma levels of principal adaptation Hormones: Cortisol, Testosterone and Triiodothyronine (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Алкор Био", XEMA Co., Ltd and DRG International Inc).

According Instruction for use sets of reagents for the determination of Testosterone in human serum or plasma ("Testosterone EIA", XEMA Co., Ltd, RF), the following normal range (nM/L) is recommended: femals <0,15÷4,6; males 20-39 yrs 9,0÷38; 40-55 yrs 6,9÷21; >55 yrs 5,9÷18,1. Resting on this repering points, Hrytsak YL et al [12] builded regressive model for calculation of normal averages for males from 20 to 69 years (Fig. 1).

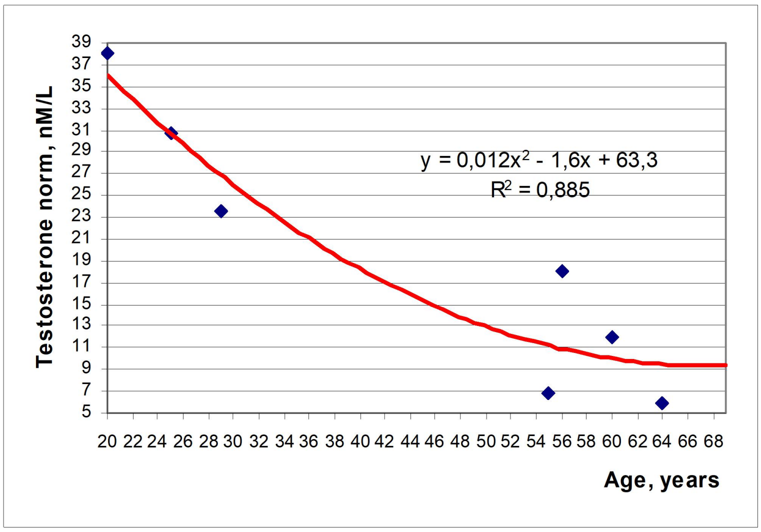


Fig. 1. Testosterone normal averages for males various age

According Instruction for use sets of reagents for the determination of Triiodothyronine in human serum or plasma ("T3 EIA", XEMA Co., Ltd, RF), is recommended the normal range 1,2÷3,2 nM/L for both female and male. Normal range Cortisol level makes 150÷660 nM/L at both men and women.

Then we estimated plasma lipide spectrum: total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of α-lipoproteins (by the enzyme method by Hiller G. [11] after precipitation of notα-lipoproteins; prae-β-lipoproteins (expected by the level of triacylglycerides, by a certain meta-periodate method); β-lipoproteins (expected by a difference between a total cholesterol and cholesterol in composition α-and prae-β-lipoproteins).

We determined also the plasma and daily urine levels of the electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (flamming photometry); nitrous metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method). The analyzes were carried out according to the instructions described in the manual [6]. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a fiery spectrophotometer "СФ-47".

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients $(Cap/Pp)^{0,5}$ and $(Pu/Cau)^{0,5}$, calcitonin by coefficients $(Cap•Pp)^{-0,5}$ and $(Cau•Pu)^{0,5}$ as well as mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$ and $(Ku/Nau)^{0,5}$, based on their classical effects and recommendations by IL Popovych [9,26].

Norms are borrowed from the Instructions and/or Database of the Truskavets' Scientific School of Balneology.

Results processed the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Adhering to the accepted algorithm HRV, endocrine and metabolic 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.

Recall that Z-scores of eleven key immune parameters were used to calculate the Immune Status Index (ISI) by the formula:

ISI=(BCCN vs St. aur.+BCCN vs E. coli+CIC+IgM+IgG+IgA+B+NK+Th+Tc+Ta)/11.

In the next phase, the profiles of HRV, endocrine and metabolic parameters were constructed together with the ISI members of the four clusters.

It was revealed (Fig. 2) that caused by balneotherapy the transformation of normal immune status into moderate immunosuppression in members (fortunately, only two) of the N/S cluster, accompanied by an increase of initially normal Baevskiy's Stress Index and markers of sympathetic tone (LFnu, LF%, AMo) and further increase in LF/HF ratio and Baevskiy's ARSI, on the one hand, in combination with a decrease in the initially normal markers of vagal tone (LF, SDNN, VLF, HF, TNN, RMSSD, HF%, MxDMn) - on the other hand, that is, there is a sympathotonic shift in the sympathetic-vagal balance. However, dramatically elevated CI and its initially normal VLF% component are reduced, and decreased ULF% and HRV entropy levels are increasing.

The absence of significant changes in the immune status of individuals with its initially normal level (N/N), however, is also accompanied by a sympathotonic shift of sympathetic-vagal balance, but much less pronounced. In this case, BARSI decreases, as does CI, and the entropy level of HRV remains stably normal.

In another immune-stable cluster (S/S), changes in HRV parameters are also minor, except that the ULF band is shifted from the lower normal to the upper.

Instead, normalization of moderate immunosuppression (N-/N) is accompanied by marked reduction of significantly increased (CI, LF/HF, BARSI, BSI) and normalization of moderately elevated (LFnu, LF%, LF) sympathicotonia markers in combination with normalization or reduction of moderately reduced vagotonia markers (MxDMn, Mode, HF%). Reduced Entropy levels of HRV are also completely normalized.

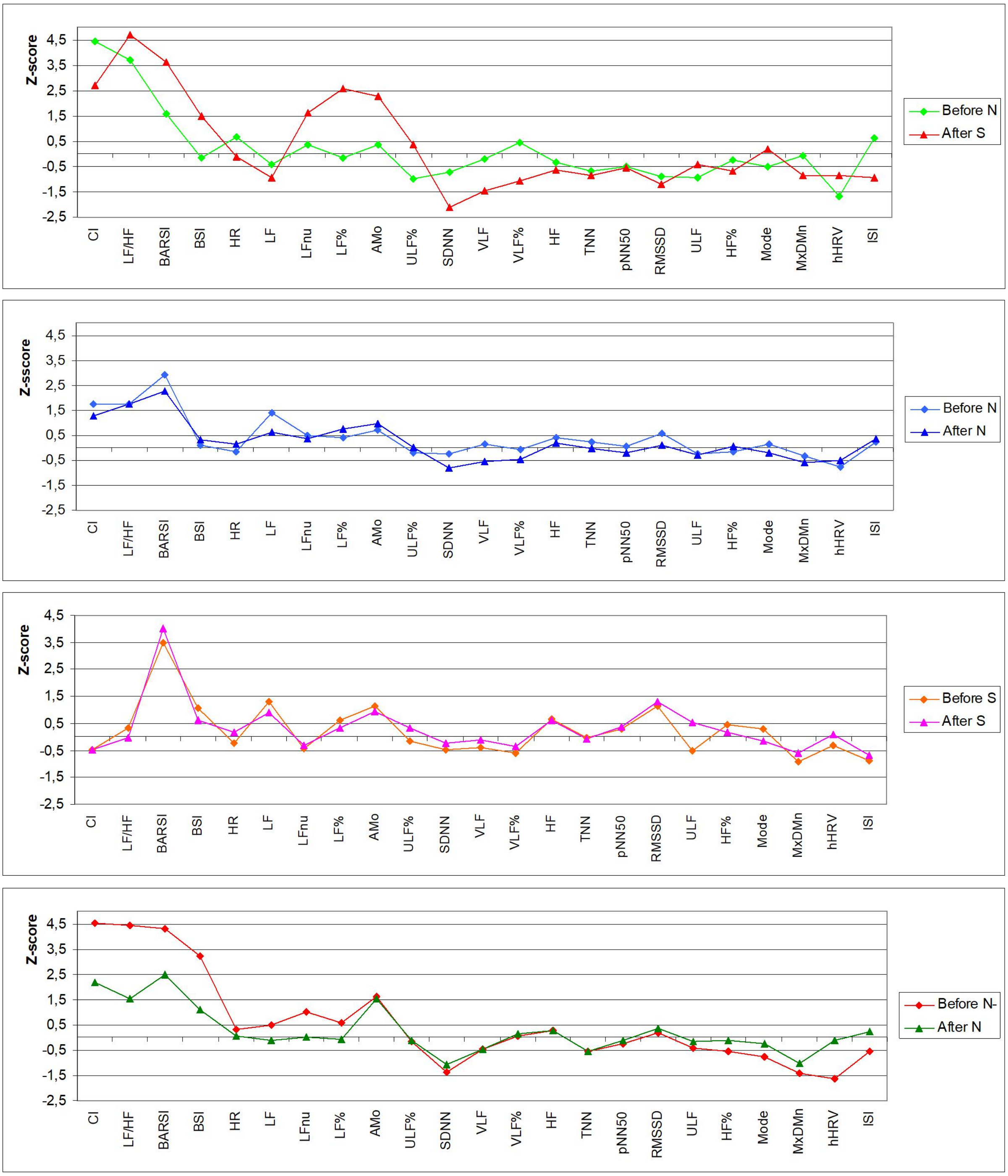


Fig. 2. Profiles of HRV parameters of members of four clusters

Regarding the endocrine support of the immunosuppressive response to balneological agents, an increase of moderately reduced levels of parathyroid and mineralocorticoid activity and T_3 to the upper zone of normal was revealed (Fig. 3), as well as an further increase in hypertestosteronemia in combination with reduction of hypercortisolemia to the upper zone of normal. The stable immunity status of N/N and S/S cluster members is, with one exception, accompanied by stable levels of registered endocrine parameters. The immuno-enhansing response is accompanied by an increase in calcitonin activity and a decrease in cortisol level.

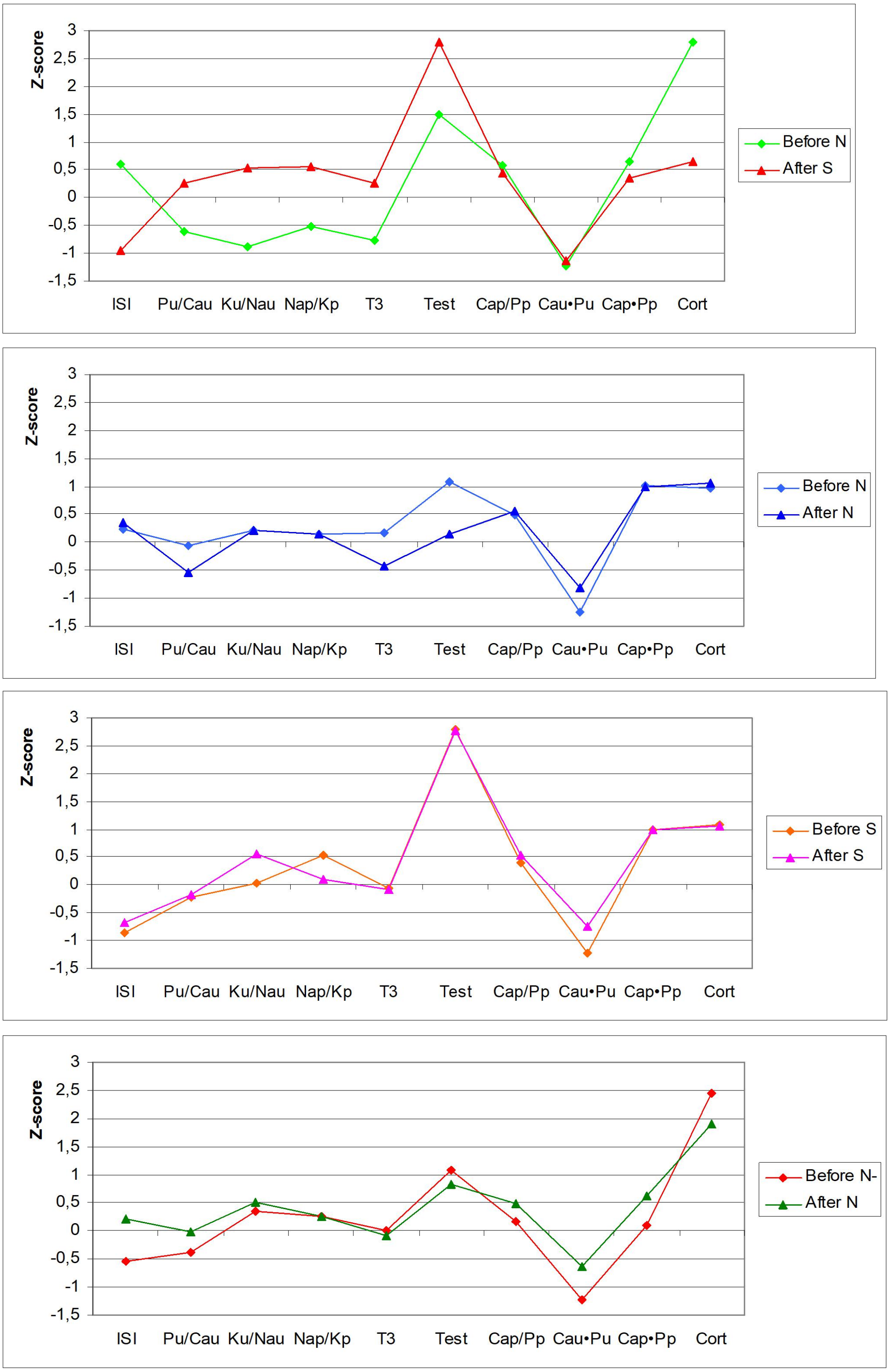


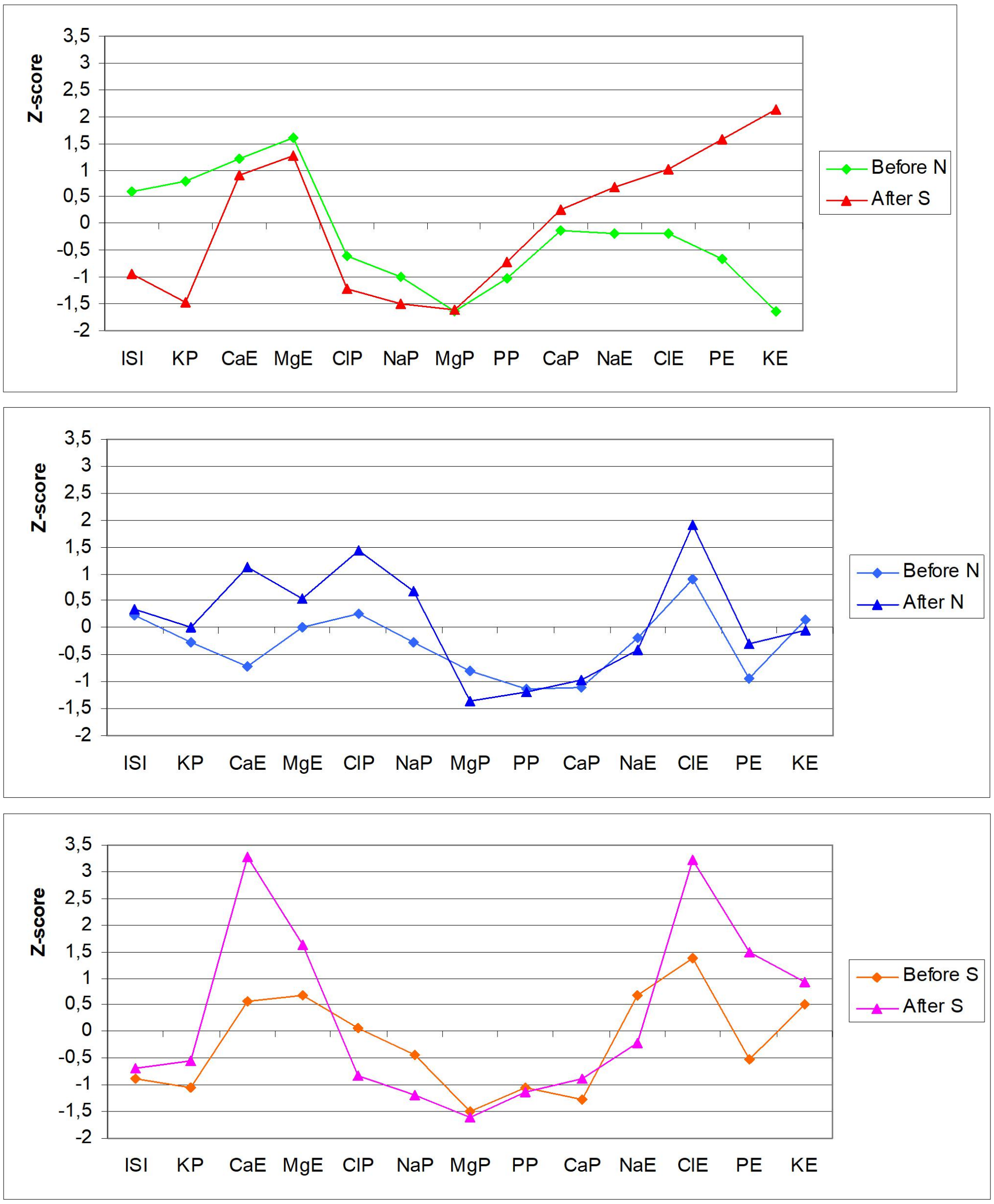
Fig. 3. Profiles of Endocrine parameters of members of four clusters

Regarding electrolyte support of immune responses, it was found (Fig. 4) that immunosuppression is accompanied by a decrease in the upper boundary level of plasma potassium and the lower boundary level of plasma chloride as well as the deepening of hyponatremia. Instead, normal levels of excretion of sodium and chloride as well as lower boundary level of phosphaturia increase, and hypokaliuria transforms into hyperkaliuria.

Stably normal immune status, however, is accompanied by an increase in the lower boundary level of calciuria, normal level of chloridemia, and increased level of chloriduria.

Stable immunosuppression resonates further with the dynamics of electrolyte exchange. In particular, upper boundary levels of magnesiumuria and, in particular, calciumuria, lower boundary levels of phosphaturia, and hyperchloriduria increase. Instead, plasma chloride and sodium levels are reduced.

The favorable dynamics of the immune status is accompanied by a significant increase in the excretion of calcium, chloride and, in particular, phosphates, as well as plasma levels of chloride and sodium in combination with a decrease in the level of phosphate.



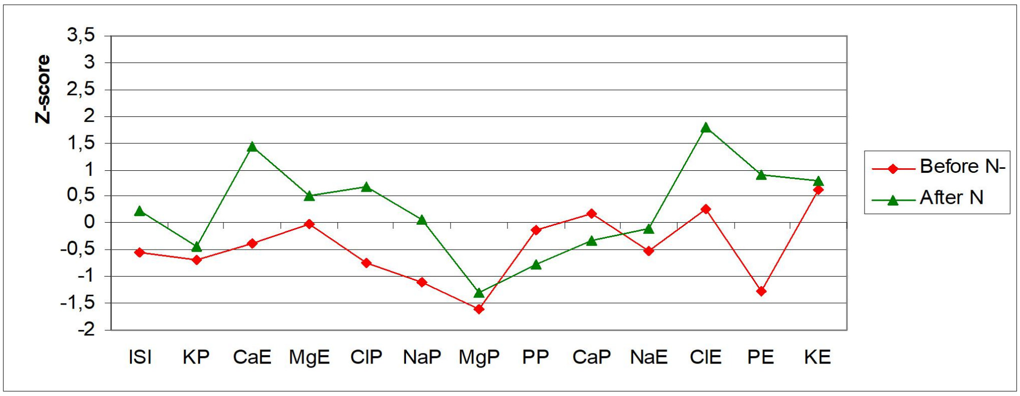
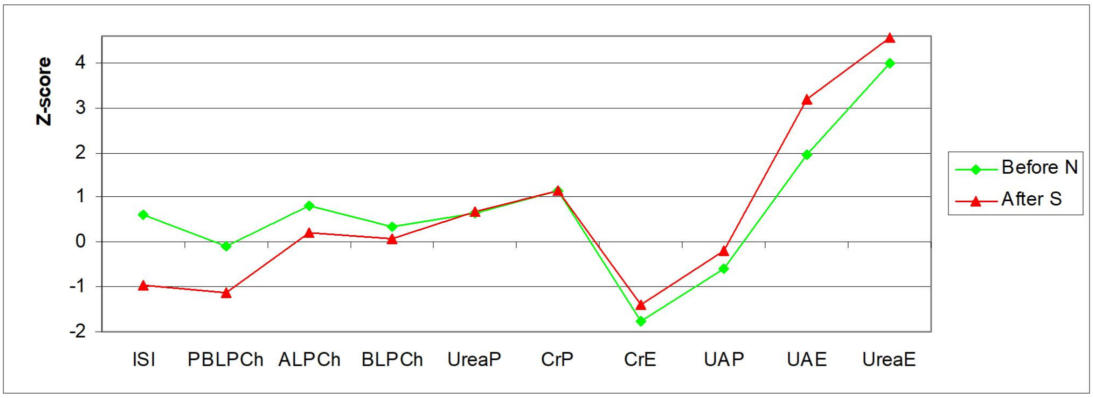


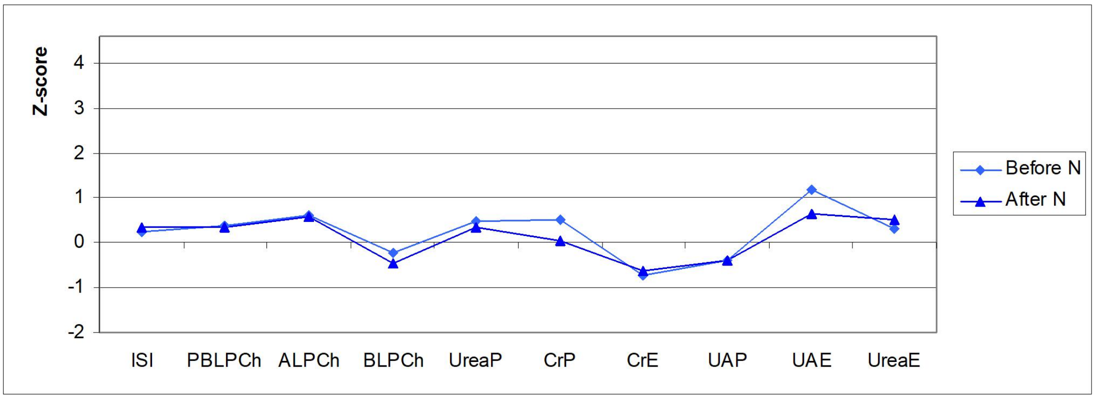
Fig. 4. Profiles of Electrolytes exchange parameters of members of four clusters

We move on to the last set of metabolic accompaniment. In this regard, it is revealed (Fig. 5) that the adverse immune response is accompanied by a decrease in both the initially normal level of very low-density lipoprotein cholesterol and the initially elevated high-density lipoprotein cholesterol. This is combined with the continued growth of hyperuricosuria.

In the absence of changes in the immune status of the members of the N/N and S/S clusters, no significant changes and metabolic accompaniment were detected, with the exception of the further increase of increased excretion of uric acid and urea in the members of the S/S cluster.

A favorable immune response is accompanied by normalization of elevated plasma levels of creatinine and urea, with reduced creatinine excretion reaching the lower area of normal, and normal urea excretion significantly increasing. To a lesser extent, this also applies to upper boundary excretion of uric acid.





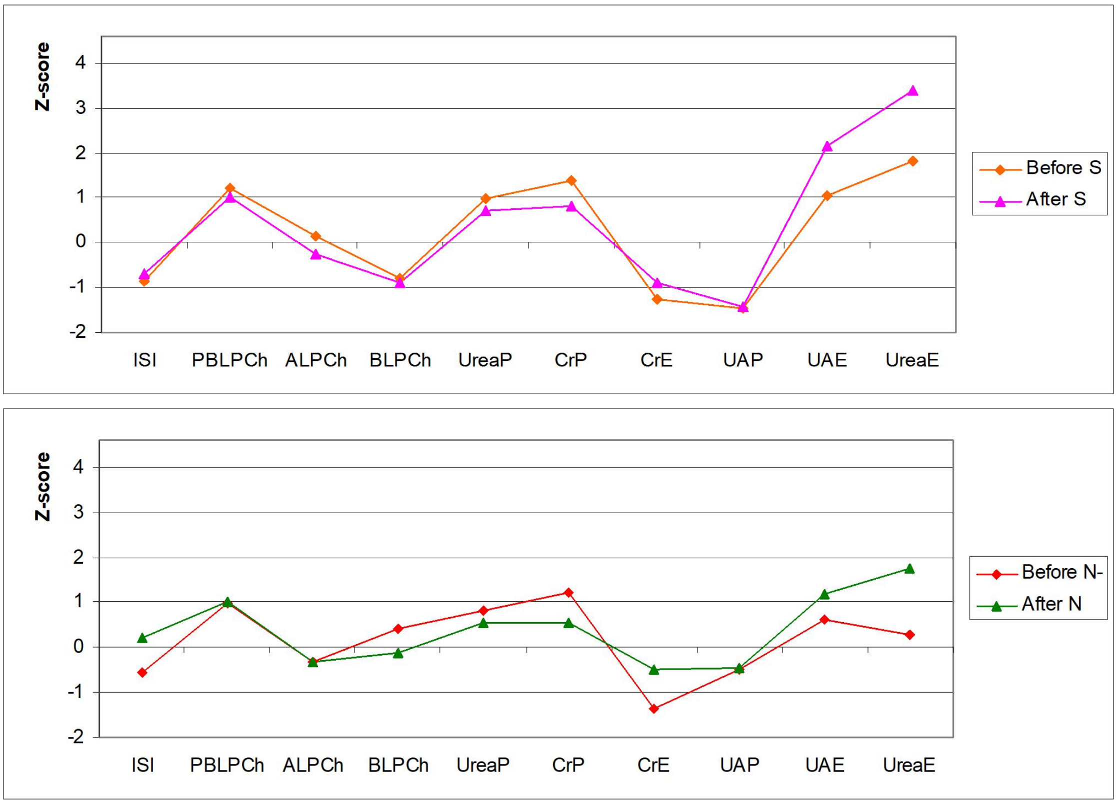


Fig. 5. Profiles of Lipid and Nitrous metabolites exchange parameters of members of four clusters

The next stage of the analysis compared changes in ISI with changes in neuro-endocrine and metabolic parameters. As a result, two patterns were created.

The largest pattern contains information about changes in 13 parameters. First of all, these are the HRV parameters that reflect the sympathetic-vagal balance (Baevskiy's ARS and Stress Indexes, LF/HF, LFnu, LF%) as well as the relative SP ULF band (0,015÷0,003 Hz).

Physiological interpretation of last need further elucidation. Discovered rhythms associated with oscillation blood level of norepinephrine (0,002 Hz) and 17-OCS (0,0019 Hz) [cit by: 16]. Apparently, the ULF band contains information on the so-called humoral channel of HRV regulation, as it is accepted with respect to Mode HRV [1].

Other components of the pattern are mineralocorticoid activity, estimated at $(Ku/Nau)^{0,5}$, excretion of potassium, as well as plasma levels of calcium, phosphate, uric acid, urea and creatinine.

The average Z-scores of changes (Mean±SE) in the listed factors in cases of suppressor immune response to balneotherapy is +1,25±0,31, while favorable changes in immunity are accompanied by a negative value of this integral parameter (-0,72±0,24). Clusters with no significant changes in ISI are accompanied by quasi-zero changes of the latter (+0,02±0,11 and -0,03±0,08 for the S/S and N/N clusters, respectively).

The alternative pattern contains information on changes in 6 parameters, namely: calcitonin activity, estimated by changes in calcium and phosphate levels in both plasma and urine; parathyroid activity estimated at $(Cap/Pp)^{0,5}$, relative SP HF band HRV as vagal tone marker as well as very low- and high-density lipoproteins cholesterol plasma levels.

The average Z-scores of changes in the listed factors in cases of unfavorable changes in immunity are accompanied by a negative value of this integral parameter (-0,40±0,16), while favorable changes in immunity are accompanied by a positive value of this integral parameter (+0,31±0,10). Clusters with no significant changes in ISI are accompanied by quasi-zero changes of the latter (-0,06±0,14 and +0,10±0,08 for the S/S and N/N clusters, respectively).

As a result, both pattern is almost linear (Fig. 6), reflecting the **downregulating**/**upregulating** immunotropic effect of the listed neuro-endocrine and metabolic factors.

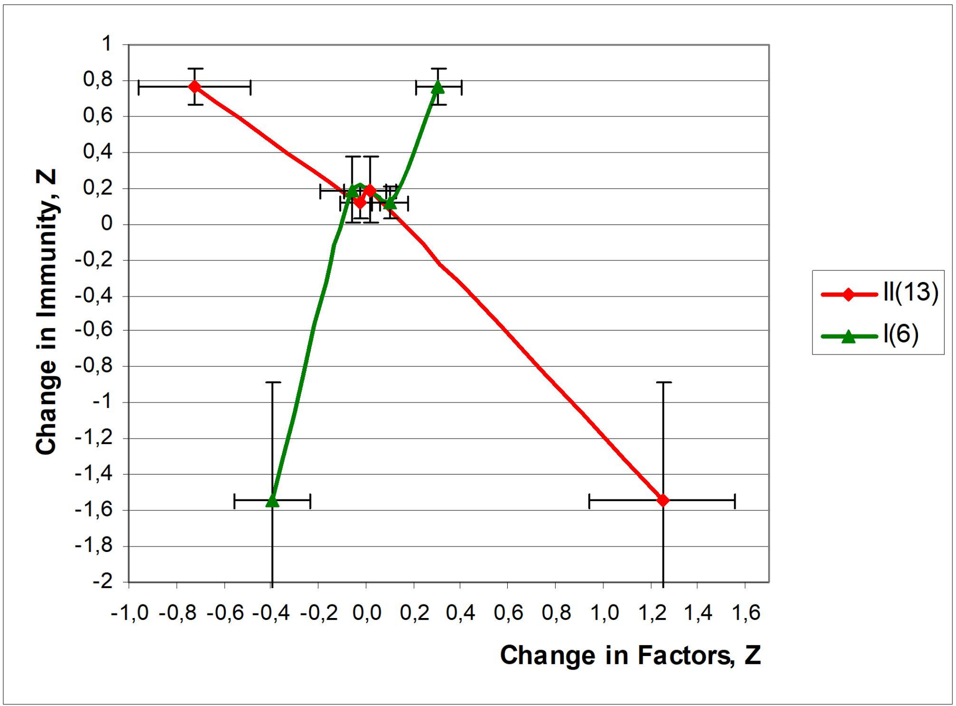


Fig. 6. Quasilinear patterns of vegetative-endocrine-metabolic accompaniments of immune responses to balneofactors

Discriminant analysis (forward stepwise method [14]) conducted to identify exactly the HRV, endocrine and metabolic parameters, in which the four immune response clusters differ significantly from each other. 25 parameters are characteristic, 12 of them related to the **HRV**, 5 to **endocrine**, 5 to **electrolytes** and 3 to **nitrous** exchange parameters (Table 1).

Table 1. Discriminant Function Analysis Summary for Changes in HRV, Endocrine and Metabolic Variables in Clusters

Step 25, N of vars in model: 25; Grouping: 4 grps;
Wilks' Λ: 0,00073; approx. $F_{(78)}$=6,0; p<10^{-6}

Variables currently in the model	Clusters of Immunity: Before/After (n)				Parameters of Wilks' Statistics					
	N/S (2)	S/S (10)	N-/N (14)	N/N (18)	Wilks Λ	Partial Λ	F-remove (3,15)	p-level	Tolerancy	Norm Cv (44)
Baevskiy's Stress Index normalized by age	-0,18 +1,47 +1,64	+0,54 -0,32 -0,86	+3,24 +1,08 -2,16	+0,10 +0,61 +0,51	,0029	,253	14,8	10^{-4}	,037	0 0,280
Baevskiy's Activity Regulatory Systems Index	1,50 3,60 +2,10	3,50 3,67 +0,17	4,31 2,31 -2,00	2,94 2,29 -0,65	,0116	,063	74,4	10^{-6}	,020	1,50 0,624
Triangulary Index HRV, units	9,5 9,1 -0,4	11,1 11,8 +0,7	9,85 9,4 -0,45	11,7 11,2 -0,5	,0019	,378	8,2	,002	,031	11,2 0,217
AMo HRV, %	42,5 62,2 +19,7	44,9 39,1 -5,8	49,3 48,7 -0,6	42,0 45,0 +3,0	,0058	,126	34,7	10^{-6}	,011	36,5 0,257
MxDMn HRV, msec	211 196 -15	207 251 +44	176 199 +24	233 219 -14	,0010	,730	1,8	,182	,019	259 0,224
Heart Rate, beats/min	73,5 67,4 -6,1	66,6 71,2 +4,5	72,7 70,5 -2,1	67,2 70,3 +3,2	,0012	,618	3,1	,059	,093	68,9 0,162
RMSSD HRV, msec	21,5 18,1 -3,4	34,6 37,7 +3,0	22,4 25,7 +3,3	30,6 26,4 -4,2	,0014	,530	4,4	,020	,038	28 0,392
(VLF+LF)/HF as Centralization Index	17,5 13,4 -4,0	6,1 6,6 +0,6	22,0 14,8 -7,2	12,0 12,3 +0,3	,0015	,482	5,4	,010	,073	8,2 0,506
ULF HRV, %	0,4 5,8 +5,4	3,6 4,7 +1,0	3,6 3,3 -0,4	3,5 4,4 +0,9	,0096	,076	60,5	10^{-6}	,012	4,3 0,926
VLF HRV, %	62,7 31,8 -30,9	41,5 47,0 +5,6	54,8 55,3 +0,4	51,7 44,2 -7,5	,0013	,566	3,8	,032	,125	53,4 0,378
Entropy HRV	0,619 0,701 0,082	0,758 0,781 0,023	0,626 0,658 0,032	0,713 0,738 0,025	,0042	,176	23,5	10^{-5}	,031	0,788 0,127
LFnu HRV, %	67,9 89,4 +21,5	61,0 64,8 +3,9	80,2 69,5 -10,6	72,3 70,8 -1,5	,0008	,915	,5	,711	,117	66 0,210
Testosterone normalized by sex&age	+1,49 +2,76 +1,27	+2,80 +2,78 -0,02	+1,07 +0,84 -0,24	+1,09 +0,14 -0,95	,0074	,099	45,7	10^{-6}	,042	0
Testosterone Plasma in males, nM/L	24,9 33,9 +9,1	28,0 27,6 -0,4	21,1 20,2 -0,9	20,4 15,2 -5,3						14,9 0,407
Testosterone Plasma in females, nM/L	lack	lack	2,55 1,98 -0,57	3,62 2,20 -1,42						2,30 0,600
Cortisol Plasma, nM/L	762 489 -274	542 540 -2	718 648 -69	528 539 +10	,0044	,165	25,3	10^{-5}	,064	405 0,524

Triiodothyronine Plasms, nM/L	1,78 2,26 +0,48	2,17 2,16 -0,01	2,20 2,15 -0,05	2,28 1,99 -0,29	,0037	,199	20,1	10^{-4}	,075	2,20 0,227
$(Pu/Cau)^{0,5}$ as Parathyroid activity	1,97 2,58 +0,61	2,25 2,28 +0,03	2,16 2,39 +0,27	2,36 2,01 -0,34	,0013	,556	4,0	,028	,033	2,40 0,294
$(Cap \bullet Pp)^{-0,5}$ as Calcitonin activity	0,67 0,64 -0,03	0,70 0,70 0,00	0,61 0,66 +0,05	0,70 0,70 0,00	,0032	,227	17,0	10^{-4}	,060	0,60 0,167
Potassium Plasma, mM/L	4,92 3,86 -1,06	4,05 4,29 +0,24	4,22 4,35 +0,12	4,42 4,56 +0,13	,0029	,249	15,1	10^{-4}	,094	4,55 0,104
Magnesium Plasma, mM/L	0,82 0,82 0,00	0,82 0,82 -0,01	0,82 0,83 +0,02	0,86 0,83 -0,03	,0030	,241	15,7	10^{-4}	,072	0,90 0,056
Calcium Excretion, mM/24h	5,50 5,21 -0,30	4,90 7,45 +2,56	4,03 5,72 +1,69	3,71 5,42 +1,72	,0021	,342	9,6	,001	,013	4,38 0,214
Phosphates Excretion, mM/24h	20,4 36,9 +16,5	21,4 36,2 +14,7	15,8 32,0 +16,1	18,3 22,9 +4,56	,0015	,499	5,0	,013	,037	25,2 0,294
Sodium Excretion, mM/24h	216 258 +42	257 215 -42	201 221 +20	216 206 -10	,0045	,164	25,5	10^{-5}	,038	225 0,211
Urea Plasma, mM/L	6,09 6,10 +0,02	6,61 6,16 -0,44	6,34 5,90 -0,44	5,79 5,58 -0,20	,0051	,144	29,8	10^{-6}	,030	5,0 0,330
Urea Excretion, mM/24h	799 846 +46	614 746 +132	481 606 +125	483 501 +18	,0056	,130	33,4	10^{-6}	,031	458 0,186
Creatinine Excretion, mM/24h	5,23 6,39 +1,16	6,85 8,05 +1,21	6,52 9,42 +2,90	8,58 8,99 +0,40	,0017	,439	6,4	,005	,121	11,0 0,300

The other registered parameters were outside the discriminatory model (Tables 2-5).

Table 2. HRV Variables currently not in the model

	Clusters of Immunity: Before/After (n)				Parameters of Wilks' Statistics					
Variables	**N/S (2)**	**S/S (10)**	**N-/N (14)**	**N/N (18)**	Wilks Λ	Parti-al Λ	F to enter	p-level	Tole-rancy	Norm Cv (44)
Mode HRV, msec	825 891 +66	900 842 -58	833 824 -10	889 850 -38	,0006	,790	,94	,468	,065	870 0,201
SDNN HRV, msec	45,5 33,4 -12,1	49,3 56,5 +7,2	38,4 42,6 +4,3	53,5 46,8 -6,7	,0006	,863	,74	,547	,039	55 0,201
pNN_{50} HRV, %	4,0 2,1 -1,9	13,6 14,2 +0,5	6,0 8,3 +2,2	11,0 7,2 -3,8	,0006	,790	1,24	,333	,041	9,2 1,518
ULF HRV, $msec^2$	8 72 +64	59 178 +118	72 93 +20	94 85 -8	,0006	,833	,94	,449	,085	122 1,021
VLF HRV, $msec^2$	1271 294 -977	1014 1443 +429	924 935 +10	1542 961 -582	,0006	,839	,90	,468	,065	1384 0,578
LF HRV, $msec^2$	518 586 +68	929 1132 +204	685 594 -92	1169 880 -288	,0005	,734	1,69	,214	,065	627 0,529
HF HRV, $msec^2$	233 69 -163	642 656 +13	223 507 +283	506 423 -82	,0007	,932	,34	,798	,022	344 1,358
LF/HF ratio	5,95 8,41 +2,46	2,79 2,88 +0,10	7,68 4,60 -3,07	4,13 4,37 +0,25	,0007	,949	,25	,859	,075	2,16 0,675
LF HRV, %	25,2 55,8 +30,6	33,9 32,6 -1,2	33,7 28,8 -4,8	31,6 35,3 +3,7						27,1 0,414
HF HRV, %	11,7 6,6 -5,1	21,0 15,7 -5,4	7,9 12,7 +4,8	13,3 16,2 +2,9						15,2 0,861

Table 3. Endocrine variables currently not in the model

Variables	Clusters of Immunity: Before/After (n)				Parameters of Wilks' Statistics					
	N/S (2)	S/S (10)	N-/N (14)	N/N (18)	Wilks Λ	Parti-al Λ	F to enter	p-level	Tole-rancy	Norm Cv (30)
$(Nap/Kp)^{0.5}$ as Mineralocorticoid activity	5,34 5,98 +0,63	5,96 5,71 -0,25	5,81 5,80 0,00	5,73 5,74 0,00	,0007	,947	,26	,854	,168	5,65 0,104
$(Ku/Nau)^{0.5}$ as Mineralocorticoid activity	0,41 0,61 +0,20	0,54 0,62 +0,07	0,59 0,61 +0,02	0,57 0,57 0,00	,0007	,953	,23	,875	,167	0,54 0,269
$(Cap/Pp)^{0.5}$ as Parathyroid activity	1,52 1,49 -0,03	1,48 1,51 +0,03	1,42 1,49 +0,07	1,50 1,51 +0,01	,0007	,932	,34	,795	,310	1,38 0,167
$(Cau•Pu)^{0.5}$ as Calcitonin activity	4,80 5,00 +0,20	4,78 5,85 +1,09	4,81 6,09 +1,28	4,75 5,69 +0,93	,0006	,792	1,22	,337	,116	7,50 0,294

Table 4. Mineral variables currently not in the model

Variables	Clusters of Immunity: Before/After (n)				Parameters of Wilks' Statistics					
	N/S (2)	S/S (10)	N-/N (14)	N/N (18)	Wilks Λ	Parti-al Λ	F to enter	p-level	Tole-rancy	Norm Cv (30)
Calcium Plasma, mM/L	2,28 2,34 +0,06	2,11 2,17 +0,06	2,33 2,25 -0,08	2,13 2,16 +0,02	,0007	,897	,53	,666	,303	2,30 0,065
Phosphate Plasma, mM/L	0,99 1,06 +0,06	0,99 0,97 -0,02	1,17 1,04 -0,13	0,97 0,96 -0,01	,0007	,970	,14	,932	,096	1,20 0,167
Chloride Plasma, mM/L	99,6 97,5 -2,1	101,7 98,8 -2,9	99,1 103,7 +4,6	102,3 106,1 +3,8	,0005	,632	2,72	,084	,225	101,5 0,032
Sodium Plasma, mM/L	140,1 137,6 -2,6	142,9 139,2 -3,7	139,5 145,3 +5,8	143,6 148,4 +4,8	,0005	,632	2,72	,084	,225	145 0,034
Magnesium Excretion, mM/24h	5,80 5,44 -0,36	4,81 5,81 +1,00	4,09 4,64 +0,55	4,11 4,68 +0,57	,0005	,707	1,93	,171	,180	4,10 0,256
Potassium Excretion, mM/24h	36 102 +66	74 81 +7	76 79 +3	67 64 -3	,0007	,922	,39	,759	,134	65 0,269
Chloride Excretion, mM/24h	162 197 +35	207 260 +53	175 219 +44	193 222 +29	,0006	,826	,98	,429	,158	167,5 0,172

Table 5. Nitrous and Lipide variables currently not in the model

	Clusters of Immunity: Before/After (n)				Parameters of Wilks' Statistics					
Nitrous and Lipide Variablesl	N/S (2)	S/S (10)	N-/N (14)	N/N (18)	Wilks Λ	Par-tial Λ	F to enter	p-le-vel	Tole-ran-cy	Norm Cv (44)
Uric Acid Plasma, μM/L	351 379 +28	228 229 +1	344 346 +2	317 317 0	,0007	,932	,34	,795	,563	366 0,181
Uric Acid Excretion, mM/24h	4,46 5,38 +0,92	3,80 4,62 +0,82	3,45 3,88 +0,42	3,89 3,47 -0,42	,0007	,909	,47	,709	,113	3,00 0,250
Creatinine Plasma, μM/L	94,9 94,3 -0,6	97,8 90,4 -7,4	93,0 84,5 -8,5	81,8 76,3 -5,6	,0007	,892	,56	,647	,353	77 0,177
Cholesterol Plasma total, mM/L	5,87 5,38 -0,49	5,21 5,09 -0,11	5,81 5,47 -0,35	5,40 5,33 -0,16	,0006	,828	,97	,435	,283	5,44 0,184
High Density Lipopro-teins Cholesterol, mM/L	1,48 1,34 -0,14	1,36 1,29 -0,07	1,28 1,28 0,0	1,52 1,51 -0,01	,0005	,644	2,58	,095	,330	1,35 0,179
Low Density Lipopro-teins Cholesterol, mM/L	3,90 3,73 -0,17	3,08 3,09+ 0,01	3,83 3,48 -0,35	3,30 3,16 -0,14	,0006	,795	1,21	,344	,344	3,56 0,179
Very Low Density Lipoproteins Chole-sterol, mM/L	0,49 0,31 -0,18	0,77 0,71 -0,06	0,70 0,70 0,00	0,58 0,57 -0,01	,0007	,909	,47	,709	,473	0,53 0,335
Klimov's Atherogenicity coefficient	4,13 3,34 -0,79	3,26 3,25 -0,01	3,86 3,52 -0,34	2,83 2,66 -0,18	,0006	,792	1,22	,338	,500	3,03 0,184

The variables are ranked by criterion Lambda (Table 6).

Table 6. Summary of Stepwise Analysis for Changes in HRV, Endocrine and Metabolic Variables in Clusters

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Testosterone normalized by sex&age	5,03	,005	,726	5,0	,005
LFnu HRV, %	3,65	,021	,567	4,3	,001
$(Pu/Cau)^{0.5}$ as Parathyroid Activity	3,12	,037	,455	3,9	10^{-3}
VLF HRV, %	2,38	,085	,381	3,6	10^{-3}
Urea Excretion, mM/24h	2,62	,066	,313	3,5	10^{-4}
$(Cap•Pp)^{-0,5}$ as Calcitonin Activity	2,94	,047	,250	3,5	10^{-4}
Baevskiy's Stress Index normalized	3,22	,035	,195	3,6	10^{-5}
Potassium Plasma, mM/L	1,76	,175	,168	3,4	10^{-5}
Baevskiy's Activity Regul Systems Ind	1,57	,215	,146	3,2	10^{-4}
AMo HRV, %	2,18	,110	,121	3,2	10^{-5}
Heart Rate, beats/min	1,77	,174	,103	3,1	10^{-5}
ULF HRV, %	2,27	,102	,083	3,2	10^{-5}
Urea plasma, mM/L	2,15	,116	,068	3,2	10^{-5}
Sodium Excretion, mM/24h	2,15	,117	,055	3,2	10^{-5}
Phosphates Excretion, mM/24h	1,79	,174	,045	3,2	10^{-5}
Creatinine Excretion, mM/24h	2,10	,126	,030	3,2	10^{-5}
Magnesium Plasma, mM/L	2,50	,085	,022	3,3	10^{-5}
Triangulary Index HRV, units	1,53	,235	,019	3,3	10^{-5}
Entropy HRV	3,06	,051	,013	3,5	10^{-6}
(VLF+LF)/HF as Centralization Index	1,95	,155	,010	3,5	10^{-6}
Cortisol Plasma, nM/L	2,47	,093	,007	3,7	10^{-6}
Triiodothyronine Plasma, nM/L	4,57	,015	,004	4,2	10^{-6}
MxDMn HRV, msec	4,21	,021	,002	4,7	10^{-6}
Calcium Excretion, mM/24h	3,71	,034	,001	5,2	10^{-6}
RMSSD HRV, msec	4,43	,020	,001	6,0	10^{-6}

Next, the 25-dimensional space of discriminant variables transforms into 3-dimensional space of canonical roots, which are a linear combination of discriminant variables. The canonical correlation coefficient is for Root 1 0,991 (Wilks' Λ=0,0007; $\chi^2_{(78)}$=202; p<10^{-6}), for Root 2 0,934 (Wilks' Λ=0,0403; $\chi^2_{(50)}$=90; p=0,0005) and for Root 3 0,827 (Wilks' Λ=0,316; $\chi^2_{(24)}$=32; p=0,121). The major root contains 86% of discriminative properties, the second 11% and the minor 3% only.

Table 7 presents standardized (normalized) and raw (actual) coefficients for discriminant variables. The calculation of the discriminant root values for each person as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each patient in the information space of the roots.

Table 7. Standardized and Raw Coefficients and Constants for changes in HRV, Endocrine and Metabolic Variables in Clusters

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Testosterone normalized by sex&age	-4,515	-1,105	-,874	-4,869	-1,191	-,942
LFnu HRV, %	,136	,835	,389	,010	,064	,030
$(Pu/Cau)^{0,5}$ as Parathyroid Activity	1,510	-3,387	-1,257	2,077	-4,659	-1,730
VLF HRV, %	1,836	-,317	,320	,122	-,021	,021
Urea Excretion, mM/24h	-5,101	-1,326	-1,350	-,025	-,007	-,007
$(Cap \bullet Pp)^{-0,5}$ as Calcitonin Activity	-3,402	-1,138	-,751	-50,32	-16,84	-11,11
Baevskiy's Stress Index normalized by age	-4,318	-,135	-1,703	-1,890	-,059	-,745
Potassium Plasma, mM/L	2,848	,010	,132	4,904	,017	,227
Baevskiy's Activity Regulatory Systems Index	-6,893	-,328	-,854	-2,921	-,139	-,362
AMo HRV, %	-8,818	,808	1,221	-,705	,065	,098
Heart Rate, beats/min	-1,852	-,214	-1,012	-,234	-,027	-,128
ULF HRV, %	-8,870	-,058	,334	-1,481	-,010	,056
Urea plasma, mM/L	-5,336	-,703	,005	-4,209	-,554	,004
Sodium Excretion, mM/24h	4,420	1,588	1,066	,045	,016	,011
Phosphates Excretion, mM/24h	-2,538	2,128	2,168	-,149	,125	,128
Creatinine Excretion, mM/24h	1,946	,375	1,067	,494	,095	,271
Magnesium Plasma, mM/L	3,103	-,914	,663	66,74	-19,67	14,27
Triangulary Index HRV, units	4,004	2,237	,031	1,133	,633	,009
Entropy HRV	5,111	-,865	-,379	42,32	-7,160	-3,136
(VLF+LF)/HF as Centralization Index	-1,755	-2,019	-,889	-,180	-,207	-,091
Cortisol Plasma, nM/L	3,626	,431	,163	,017	,002	,001
Triiodothyronine Plasma, nM/L	3,254	,046	,653	5,646	,080	1,133
MxDMn HRV, msec	-2,729	-1,486	-2,738	-,041	-,022	-,041
Calcium Excretion, mM/24h	6,767	-2,180	-1,347	1,692	-,545	-,337
RMSSD HRV, msec	-3,335	,359	1,377	-,235	,025	,097
	Constants			-2,075	-,487	-,764
	Cumulative Properties			,857	,966	1,000

Table 8 shows the correlation coefficients of changes in discriminant variables with canonical discriminant roots, the cluster centroids of roots, as well as the changes in normalized values of the discriminant variables.

Table 8. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of changes in HRV, Endocrine and Metabolic Variables for Clusters

	Correlations Variables-Roots			N/S (2)	S/S (10)	N-/N (14)	N/N (18)
Root 1 (86%)	**R 1**	**R 2**	**R 3**	**-29,6**	**-3,6**	+2,7	+3,2
Testosterone level normalized by sex&age	**-,069**	-,127	,065	**+1,27**	**-0,02**	-0,24	-0,95
Males (n=2+10+12+10)				**+1,27**	**-0,02**	-0,21	-0,90
Females (n=0+ 0+ 2+ 8)				**lack**	**lack**	-0,41	-1,03
LFnu HRV	**-,062**	,075	-,116	**+1,25**	**+0,29**	-0,79	-0,12
Baevskiy's Activity Regulatory Systems Ind	**-,043**	,057	-,108	**+2,10**	**+0,17**	-2,00	-0,65
Triiodothyronine Plasma	**-,037**	-,063	,050	**+1,03**	**-0,02**	-0,10	-0,58
$(Pu/Cau)^{0.5}$ as Parathyroid Activity	**-,030**	-,112	,150	**+0,86**	+0,04	+0,38	-0,49
ULF band HRV relative SPD	**-,023**	,032	-,007	**+1,29**	**+0,24**	-0,10	+0,22
Phosphates Excretion	**-,017**	-,114	,062	**+2,23**	+1,99	+2,18	+0,62
Entropy HRV	**-,013**	,012	,018	**+0,82**	+0,23	+0,32	+0,24
Potassium Plasma	**,048**	-,033	-,190	**-2,27**	**+0,52**	+0,26	+0,29
VLF band HRV relative SPD	**,041**	-,138	-,094	**-1,53**	**+0,28**	+0,02	-0,37
Cortisol Plasma	**,030**	,019	-,140	**-2,14**	**-0,01**	-0,54	+0,08
$(Cap\bullet Pp)^{-0,5}$ as Calcitonin Activity	**,023**	-,102	,159	**-0,28**	**-0,01**	+0,51	-0,02
Calcium Excretion	**,010**	-,028	-,075	**-0,32**	**+2,73**	+1,80	+1,83
Root 2 (11%)	**R 1**	**R 2**	**R 3**	+2,76	-2,45	**-2,15**	**+2,73**
Magnesium Plasma	-,009	**-,132**	,158	+0,04	-0,12	**+0,31**	**-0,55**
MxDMn HRV	-,001	**-,113**	-,026	-0,76	+0,77	**+0,43**	**-0,25**
Urea Excretion	-,004	**-,107**	,007	+0,54	+1,55	**+1,47**	**+0,21**
Creatinine Excretion	,002	**-,084**	,121	+0,35	+0,37	**+0,88**	**+0,12**
RMSSD HRV	,000	**-,084**	,024	-0,31	+0,41	**-0,08**	**-0,47**
Triangulary Index HRV	-,004	**-,023**	-,045	-0,16	+0,27	**-0,19**	**-0,22**
Baevskiy's Stress Index normalized by age	-,017	**,144**	-,094	+1,64	-0,86	**-2,16**	**+0,51**
AMo HRV	-,033	**,096**	,111	+1,96	-0,66	**-0,04**	**+0,29**
Urea plasma	-,005	**,039**	,003	+0,01	-0,34	**-0,27**	**-0,12**
Root 3 (3%)	**R 1**	**R 2**	**R 3**	+1,97	**-2,07**	+1,57	-0,29
Heart Rate	,022	,033	**-,192**	-0,77	**+0,39**	-0,18	+0,29
(VLF+LF)/HF as Centralization Index	,003	,056	**-,144**	-1,71	**+0,18**	-2,11	-0,49
Sodium Excretion	-,004	,005	**,179**	+0,88	**-0,87**	+0,42	-0,22

Extreme left localization along the axis of the first root of members of the **N/S** cluster (Figs. 7 and 8) reflects their maximally increase in parameters that correlate with the root **negatively**, and maximally decrease in parameters that correlate with the root **positively**. Instead, the extreme right localization of the **N-/N** and **N/N** clusters members reflects the maximum decrease/increase in the same parameters. The members of both clusters do not differ in the totality of the parameters listed (mixed along the axis of the first root). The intermediate position is taken by the members of the **S/S** cluster.

Instead, the members of the **N-/N** and **N/N** clusters are clearly distinguished along the axis of the second root (Figs. 7 and 9). Lower localization of cluster **N-/N** than cluster **N/N** reflects an increase (or no change) as opposed to a decrease in parameters that correlate with the root **negatively**, and vice versa, a decrease as opposed to an increase (or no change) in parameters that correlate with the root **positively**. Members of the **S/S** cluster occupy a similar axis zone.

According to another constellation of parameters, **S/S** cluster are delimited also along the axis of the third root (Fig. 10 and 11).

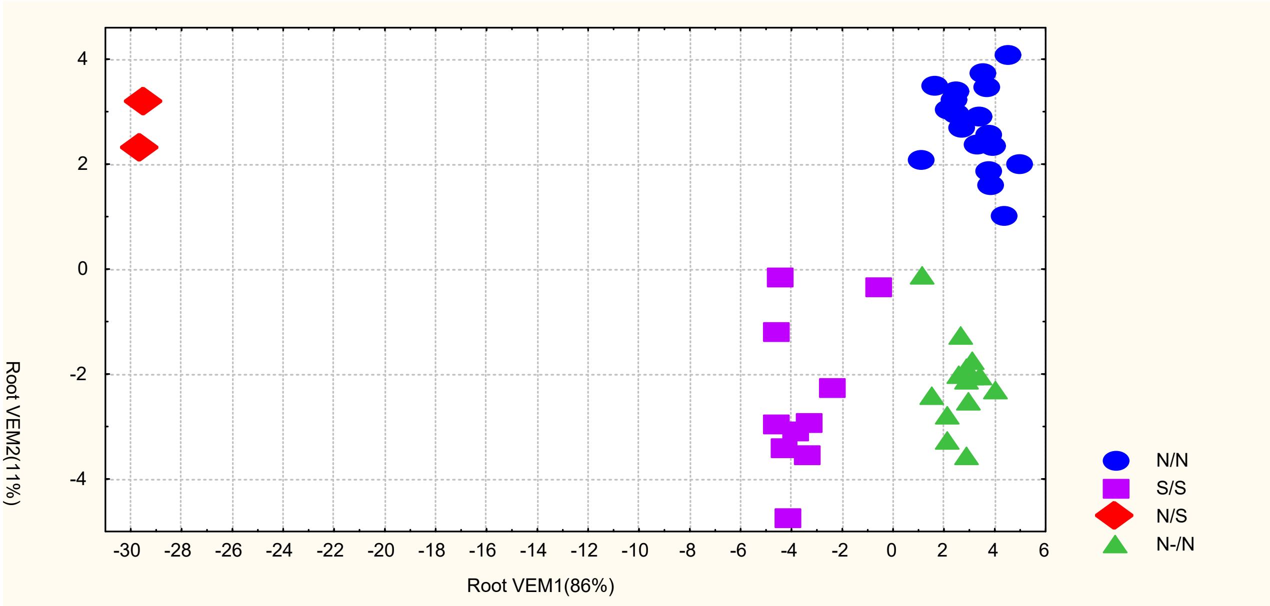


Fig. 7. Scatterplot of individual values of the first and second roots in which condensed information about of the changes in HRV, endocrine and metabolic parameters of the members of the four clusters

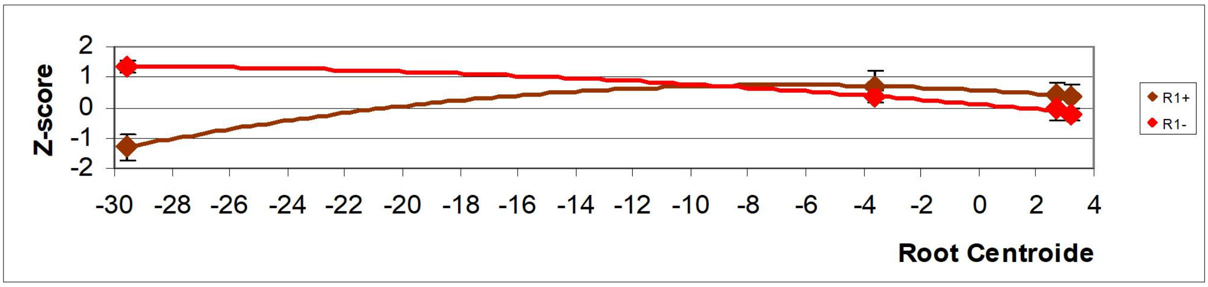


Fig. 8. Patterns of changes in HRV, Endocrine and Metabolic parameters, the information of which is condensed in the first root

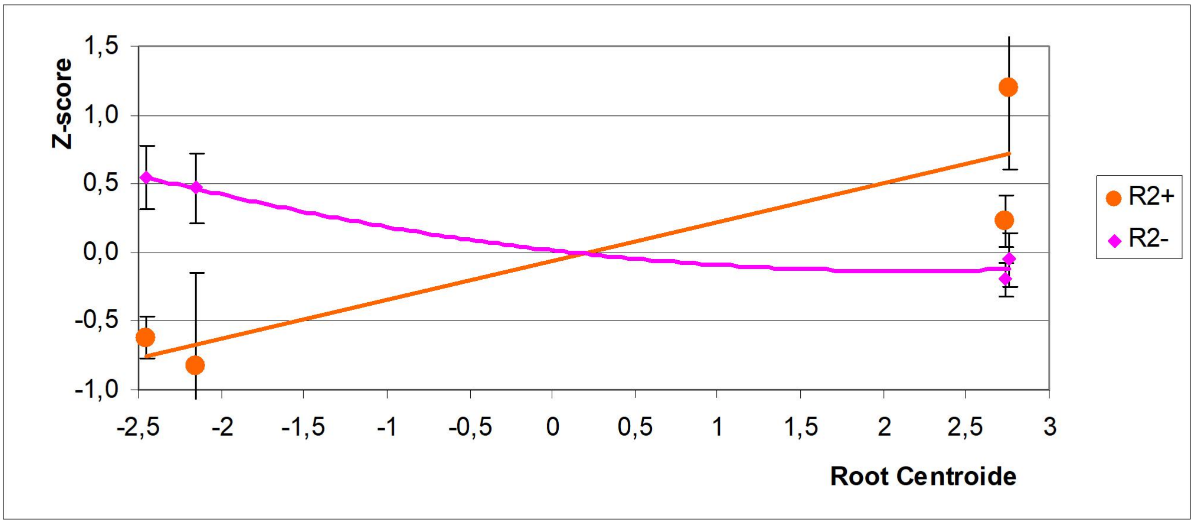


Fig. 9. Patterns of changes in HRV, Endocrine and Metabolic parameters, the information of which is condensed in the second root

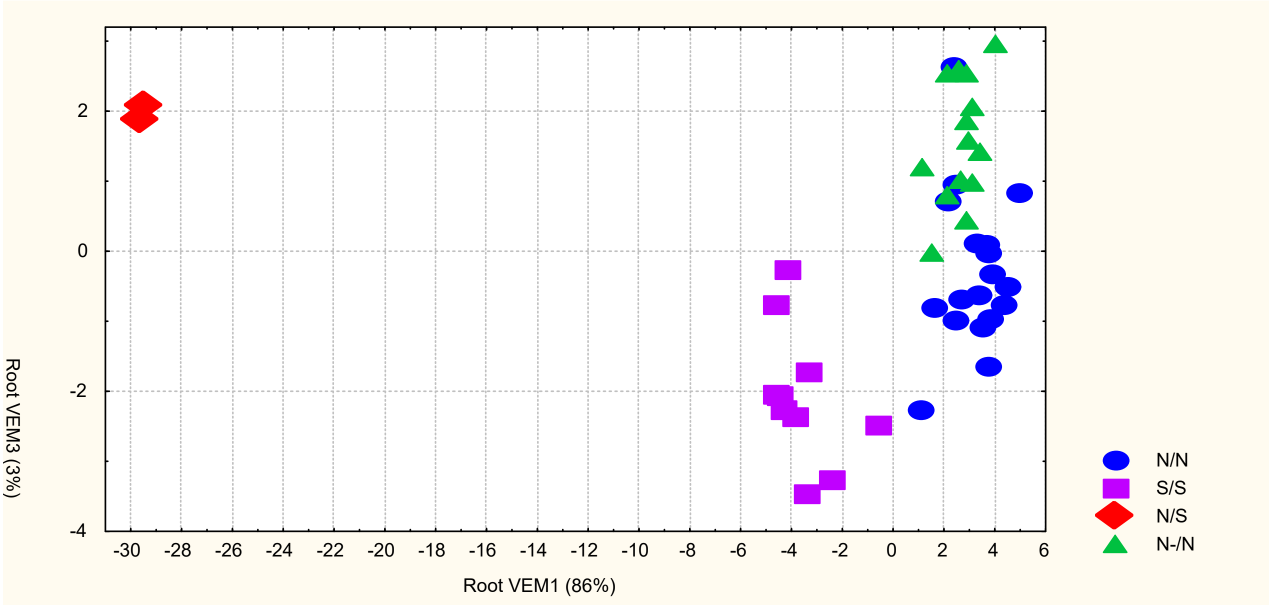


Fig. 10. Scatterplot of individual values of the first and third roots in which condensed information about of the changes in HRVs, endocrine and metabolic parameters of the members of the four clusters

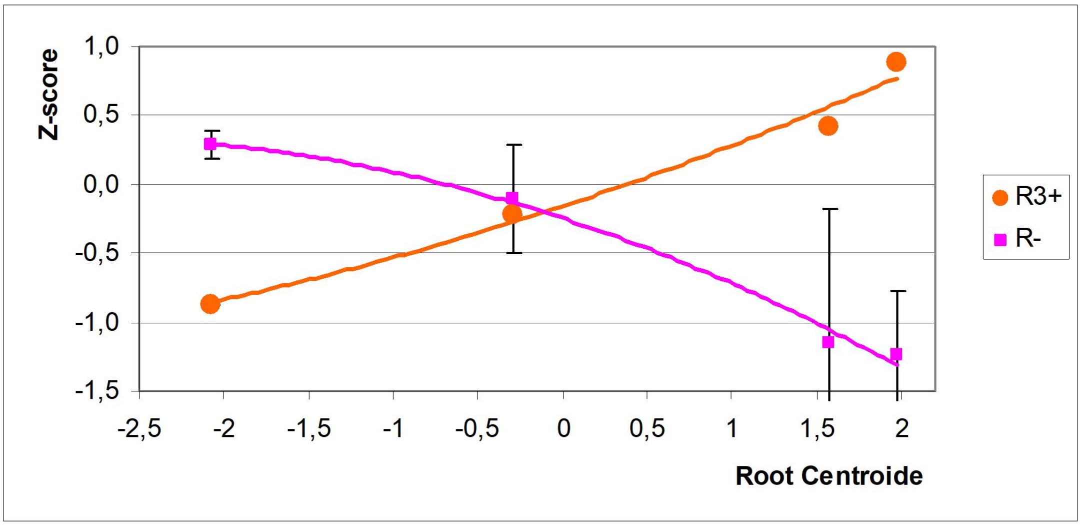


Fig. 11. Patterns of changes in HRV, Endocrine and Metabolic parameters, the information of which is condensed in the third root

In general, all four clusters on the planes of the discriminant roots are quite clearly delineated, which is documented by calculating the Mahalanobis distances (Table 9).

Table 9. Squared Mahalanobis Distances between Clusters, F-values (df=26,2) and p-levels

Clusters	N/N	S/S	N/S	N-/N
N/N	0	83	1184	30
S/S	**7,1** 10^{-4}	0	789	58
N/S	**16,1** 10^{-6}	**10,2** 10^{-4}	0	1170
N-/N	**3,2** 0,011	**4,4** 0,002	**15,7** 10^{-6}	0

The same discriminant parameters can be used to identify the belonging of one or another person to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying functions (Table 10).

Table 10. Coefficients and Constants for Classification Functions of Clusters

Clusters	N/N	S/S	N/S	N-/N
Variables	p=,409	p=,227	p=,045	p=,318
Testosterone normalized by sex&age	-30,71	10,06	126,5	-24,29
LFnu HRV, %	,152	-,300	-,118	-,108
$(Pu/Cau)^{0.5}$ as Parathyroid Activity	-4,369	8,772	-76,40	14,09
VLF HRV, %	,486	-,270	-3,469	,569
Urea Excretion, mM/24h	-,153	,065	,662	-,121
$(Cap•Pp)^{-0,5}$ as Calcitonin Activity	-322,6	124,6	1299	-236,7
Baevskiy's Stress Index normalized by age	-11,00	3,414	49,17	-11,18
Potassium Plasma, mM/L	25,15	-8,512	-134,8	23,10
Baevskiy's Activity Regulatory Systems Index	-15,93	5,188	78,84	-14,50
AMo HRV, %	-3,575	,685	19,72	-3,364
Heart Rate, beats/min	-1,484	,462	5,871	-1,476
ULF HRV, %	-7,842	2,130	40,77	-6,967
Urea plasma, mM/L	-24,99	6,340	112,8	-20,23
Sodium Excretion, mM/24h	,285	-,123	-1,167	,204
Phosphates Excretion, mM/24h	-,280	-,144	4,906	-,579
Creatinine Excretion, mM/24h	2,875	-1,436	-12,66	2,676
Magnesium Plasma, mM/L	305,5	-69,45	-1847	395,3
Triangulary Index HRV, units	8,275	-2,683	-28,775	4,655
Entropy HRV	190,3	-53,32	-1202	198,7
(VLF+LF)/HF as Centralization Index, units	-1,555	,893	4,115	-,630
Cortisol Plasma, nM/L	,097	-,028	-,450	,080
Triiodothyronine Plasma, nM/L	29,78	-10,83	-152,4	28,75
MxDMn HRV, msec	-,229	,238	1,022	-,177
Calcium Excretion, mM/24h	7,273	-,754	-48,90	8,476
RMSSD HRV, msec	-1,170	,118	6,751	-,997
Constants	-21,20	-6,626	-390,8	-17,84

We can retrospectively recognize members of all clusters unmistakably (Table 11).

Table 11. Classification Matrix for Clusters
Rows: Observed classifications; Columns: Predicted classifications

Clusters	Percent correct	N/N p=,409	S/S p=,227	N/S p=,045	N-/N p=,318
N/N	100	**18**	0	0	0
S/S	100	0	**10**	0	0
N/S	100	0	0	**2**	0
N-/N	100	0	0	0	**14**
Total	100	18	10	2	14

CONCLUSION

Individual immune responses to adaptogenic balneofactors are accompanied by characteristic changes in the parameters of the autonomic nervous and endocrine systems, as well as the exchange of electrolytes, nitrogen metabolites and lipids.

A detailed analysis of the neuro-endocrine and metabolic companions and predictors of individual immune responses, as well as sexual dimorphism, will be topics in subsequent publications.

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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.

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