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RELATIONSHIPS BETWEEN CHANGES IN ENTROPY OF THE EEG AND PARAMETERS OF THE IMMUNITY

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Abstract

Background. Previously, we have shown that the entropy of the normalized parameters of the HRV and spectral power density (SPD) of loci of EEG significantly correlate with the entropy and parameters of immunity, which testifies to their modulating regulatory effects. The purpose of this study is to analyze the relationships between **changes** in entropy and immunity under the influence of natural adaptogens. **Material and methods.** In basal conditions in 37 men and 14 women with chronic pyelonephritis and cholecystitis in remission as well as without clinical diagnose but with dysfunction of neuro-endocrine-immune complex and metabolism, we recorded twice, before and after balneotherapy at the spa Truskavets', EEG ("NeuroCom Standard") and HRV ("Cardiolab+VSR"). Than we evaluated immune status on a set of I and II levels recommended by the WHO. The Entropy of normalized SPD for each locus of EEG and HRV as well as Immunocytogram and Leukocytogram calculated using Shannon's formula. **Results.** Preliminary analysis revealed different orientation of entropy changes in patients, so three clusters were created. Balneotherapy has a generalized negentropic effect on EEG of 2/3 patients. On the other hand, the members of the other two clusters have substantially increased EEG entropy overall, but there are significant differences with respect to individual loci. The immunotropic effects of balneotherapy are unrelated to changes in integral entropy of EEG. As a result of discriminant analysis were selected as characteristic entropy changes at only 9 loci out of 16, accompanied by changes in 10 partial parameters of immunity and integral immune index, as well as Popovych's Leukocytogram Strain Index-2. **Conclusion**. Balneotherapy causes multivariate entropy changes of individual EEG loci, conditioned by a number of predictors. This is accompanied by characteristic changes in certain parameters of immunity in line with the concept of immune homunculus.

Key words: EEG, HRV, Immunity, Entropy, Relationships, Balneotherapy.

INTRODUCTION

Previously, we have shown that in patients with chronic pyelonephritis and cholecystitis in remission as well as without clinical diagnose but with dysfunction of neuro-endocrine-immune complex and metabolism entropy of the relative (normalized) parameters of the HRV and SPD of loci of EEG significantly correlate with the entropy and parameters of immunity, which testifies to their modulating regulatory effects [7,19-21,44].

The purpose of this study is to analyze the relationships between **changes** in entropy and immunity under the influence of balneotherapeutic factors. The choice of the latter is due to their ability as natural adaptogens to exert a modulatory effect on the neuroendocrine-immune complex [9-13,15,23,24,26-28]. IL Popovych [26] advanced conception about stresslimiting adaptogene mechanism of biological and curative activity of Naftussya Water that including participation of nervous, endocrine and immune systems closely interacting in the bounds of neuroendocrine-immune complex

MATERIAL AND METHODS

The object of observation were 37 men and 14 women aged 23-76 years old, who came to the Truskavets' spa (Ukraine) for the treatment of chronic pyelonephritis and cholecystitis in remission as well as without clinical diagnose but with dysfunction of neuroendocrine-immune complex and metabolism. The survey was conducted twice, before and after standard balneotherapy (drinking bioactive water Naftussya three times a day, ozokerite applications, mineral baths every other day for 7-10 days) [28].

We recorded electrocardiogram in II lead (hardware-software complex "CardioLab+HRV" produced by "KhAI-MEDICA", Kharkiv, Ukraine) to assess the parameters of heart rate variability (HRV). For further analysis (Frequency Domain Methods) were selected 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) [1,3,8]. Simultaneosly we recorded EEG (hardware-software complex "NeuroCom Standard", KhAI Medica, Kharkiv, Ukraine) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on the tassels of ears. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%), coefficient of asymmetry (%) as well as absolute (μV^2/Hz) and relative (%) spectral power density (SPD) in the standard frequency bands: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device.

We calculated also for HRV and each locus EEG the Entropy (h) of normalized SPD using adapted formula [25,43] based on classical CE Shannon's formula [35]:

$$hHRV=[SPDHF \bullet \log_2 SPDHF+SPDLF \bullet \log_2 SPDLF+SPDVLF \bullet \log_2 SPDVLF+SPDULF \bullet \log_2 SPDULF]/\log_2 4$$

$$hEEG = - [SPD\alpha \bullet \log_2 SPD\alpha+SPD\beta \bullet \log_2 SPD\beta+SPD\theta \bullet \log_2 SPD\theta+SPD\delta \bullet \log_2 SPD\delta]/\log_2 4$$

In portion of capillary blood we counted up Leukocytogram (LCG) (Eosinophils, Stub and Segmentonucleary Neutrophils, Lymphocytes and Monocytes) and calculated two variants of Adaptation Index as well as two variants of Strain Index by IL Popovych [2,15,18].

$$\text{Strain Index-1} = [(Eo/3,5-1)^2 + (SN/3,5-1)^2 + (Mon/5,5-1)^2 + (Leu/6-1)^2]/4$$

Strain Index-2 = $[(Eo/2,75-1)^2 + (SN/4,25-1)^2 + (Mon/6-1)^2 + (Leu/5-1)^2]/4$

Immune status evaluated on a set of I and II levels recommended by the WHO. 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 ("active" T Lymphocytes) determined by test of active rosette formation. The state of humoral immunity judged by the concentration in serum of Immunoglobulins classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method).

We calculated also the Entropy (h) of Immunocytogram (ICG) and LCG using similar formulas:

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

$hLCG = - [Lymph \bullet \log_2 Lymph + Mon \bullet \log_2 Mon + Eos \bullet \log_2 Eos + SNN \bullet \log_2 SNN + StubN \bullet \log_2 StubN]/\log_2 5$

Parameters of phagocytic function of neutrophils estimated as described by MM Kovbasnyuk [29]. 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 as percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index; intensity as number of microbes absorbed one phagocytes - Microbial Count (MC) or Right's Index; completeness as percentage of dead microbes - Killing Index (KI). Based of these parameters were calculated the Bactericidity of Neutrophils (BCN), contained in 1 L of blood, by formula [28]:

BCN (10^9 Bacteras/L) = Leuk(10^9/L)•Neutrophils (%)•PhI (%)•MC (B/Phag)•KI (%)/10^4

Eleven key immune parameters were used to calculate the Immune Status Index (ISI) by the formula:

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

Results processed using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Preliminary analysis revealed different orientation of entropy changes in patients [22], so three groups were created, significantly different from each other in terms of entropy changes, while the differences between the members of each group were much smaller.

In Fig. 1 shows the profiles of changes in the normalized entropy values for individuals of different clusters.

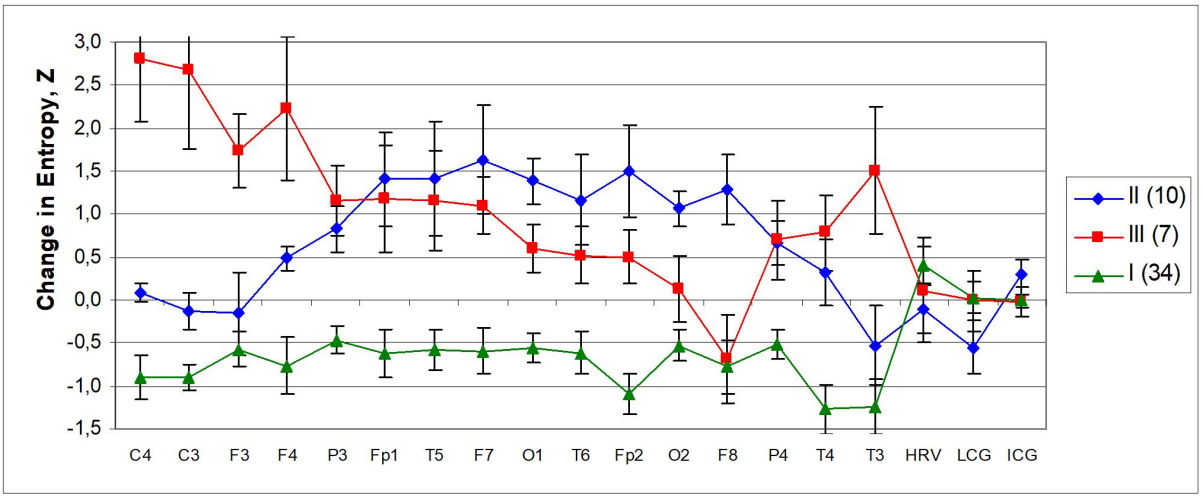

Fig. 1. Z-scores (M±SE) of changes in the entropy of SPD in loci of EEG as well as of HRV, LCG and ICG in members of different clusters

As can be seen, individuals in the major **first cluster** (66,7% of the cohort) are characterized by a moderate and approximately equal decrease in SPD entropy at all EEG loci in the absence of significant changes in HRV, ICG, and LCG entropy.

In individuals in the **second cluster** (19,6% of the cohort), the scope for the absence of significant entropy changes in HRV, ICG and LCG is supplemented by loci C4, C3, F3, F4, T4 and T3, and in the other 10 loci the entropy level is moderately increased.

In members of the **third cluster** (13,7% of the cohort), with the similar entropy stability of HRV, ICG and LCG, balneotherapy does not significantly affect the entropy of SPD at F8 and O2 loci, increasing it at Fp2, T6, O1 loci to a lesser extent than in the second. clusters, at the F7, T5, Fp1, P3, P4, and T4 loci are almost similar, and at the T3, F4, F3, C3, and C4 loci are much more pronounced.

Therefore, balneotherapy has a generalized **negentropic** effect on EEG of 2/3 patients. On the other hand, the members of the other two clusters have substantially increased EEG entropy overall, but there are significant differences with respect to individual loci. The integral **proentropic** effect of balneotherapy is greater in the members of the **third** cluster, but insignificantly. The entropy changes of HRV, ICG, and LCG are within ±0,5 σ, which we consider to be insignificant (Fig. 2).

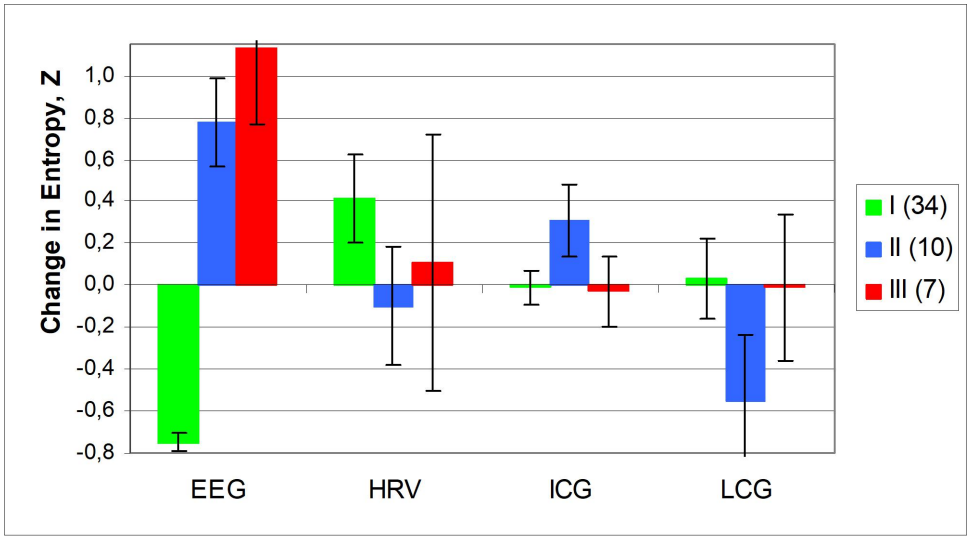

Fig. 2. Changes in normalized entropy of SPD of loci of EEG, HRV, Immunocytogram and Leukocytogram in members of different clusters

In view of the previously identified links between the parameters of EEG entropy and immunity [21] , the question is, do entropy changes affect the body's immune status? To find out, we compare the normalized profiles of integral entropy and the parameters of immunity before and after balneotherapy (Fig. 3).

As we can see, in the members of the first cluster, the complete normalization of the upper boundary level of integral EEG entropy is accompanied by the normalization of the substantially reduced bactericidal ability of neutrophils against to both types of microbes. In this case, moderately elevated levels of B lymphocytes and IgM are almost unchanged, and other parameters of immunity remain stably normal. Instead, the opposite entropy effect of balneotherapy, namely, moving its level from the mid-normal zone to the upper boundary, is accompanied by a decrease in the initially normal levels of IgA, monocytes, and entropy of the leukocytogram, as well as an initially elevated level of eosinophils. Instead, the upper boundary level of B lymphocytes becomes even higher in the absence of significant changes in other immune parameters, irrespective of their initial levels. In the members of the third cluster complete normalization of the initial negentropy is accompanied by a normalizing decrease in the increased levels of IgA and bactericidal ability of neutrophils against E. coli, on the one hand, and a decrease in the normal levels of monocytes and bactericidity of neutrophils against Staph. aureus - on the other hand. However, the level of eosinophils rises from the lower normal zone to the upper, and moderately elevated levels of IgM and B lymphocytes become even higher.

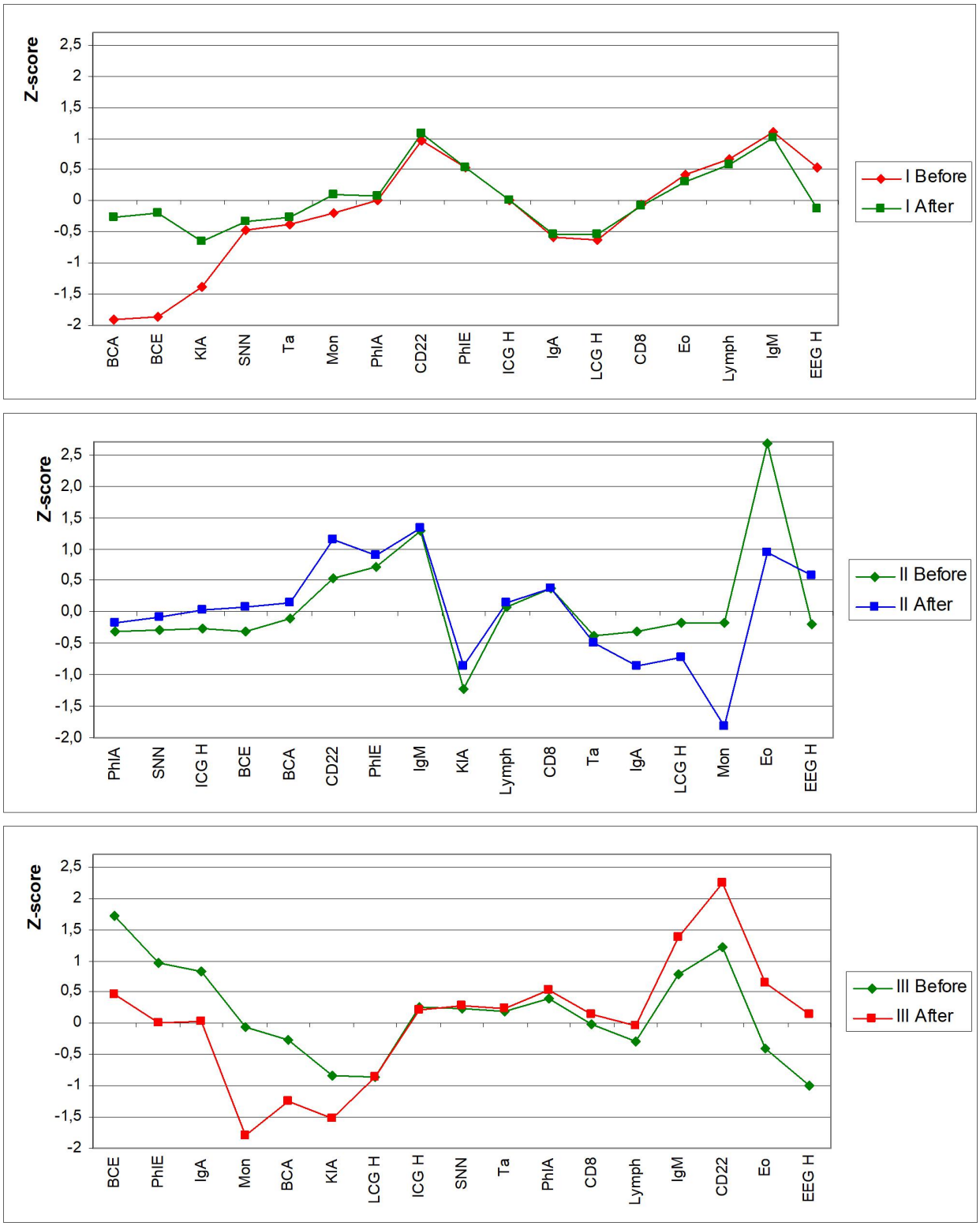

Fig. 3. Profiles of normalized levels of integral EEG entropy and parameters of immunity in members of different clusters before and after balneotherapy

It seems that the immunotropic effects of balneotherapy are unrelated to changes in integral entropy of EEG. Therefore, a discriminant analysis [14] was subsequently applied to identify,

firstly, those loci whose entropy changes differ clusters from each other, and second, constellations of immune parameters that change are characteristic of each cluster.

The program selected as characteristic entropy changes at only 9 loci out of 16, accompanied by changes in 10 partial parameters of immunity and integral immune index, as well as Popovych's Leukocytogram Strain Index-2. Interestingly, the entropies of immunocytogram, leukocytogram, and HRV were outside the discriminatory model (Tables 1 and 2).

Table 1. Discriminant Function Analysis Summary for Changes in Variables of Entropy and Immunity in Clusters

Step 21, N of vars in model: 21; Grouping: 3 grps

Wilks' Lambda: 0,0187; approx. $F_{(42)}=8,4$; $p<10^{-6}$

Variables currently in the model	**Cluster No.2 (10)**	**Cluster No. 3 (7)**	**Cluster No.1 (34)**	Wilks' Λ	Partial Λ	F-remove 2,28	p-level	Tolerancy
O1H	**+0,251**	+0,108	-0,071	,025	,736	5,0	,014	,331
P3H	**+0,103**	+0,144	-0,039	,033	,559	11,0	,0003	,337
O2H	**+0,191**	+0,022	-0,071	,029	,641	7,8	,002	,230
F7H	**+0,262**	+0,176	-0,060	,034	,550	11,5	,0002	,264
T4H	**+0,038**	+0,093	-0,127	,024	,763	4,4	,023	,536
T6H	**+0,172**	+0,077	-0,066	,029	,637	8,0	,002	,175
F8H	**+0,220**	-0,117	-0,111	,034	,546	11,6	,0002	,182
Phagocytose Ind vs Staph. aur., %	**+0,23**	+0,24	+0,10	,023	,805	3,4	,048	,475
Immune Status Index-11	+0,11	-0,16	**+0,41**	,035	,541	11,9	,0002	,060
Leukocytes, 10^9/L	-0,33	-0,42	**+0,13**	,025	,747	4,7	,017	,227
Stub Neutrophils, %	-0,10	-0,07	**+0,35**	,052	,360	24,9	10^{-5}	,124
$CD3^+$ T active Lymphocytes, %	-0,6	+0,1	**+0,7**	,030	,625	8,4	,001	,356
C3H	-0,012	**+0,253**	-0,065	,034	,549	11,5	,0002	,395
T3H	-0,055	**+0,156**	-0,111	,030	,622	8,5	,001	,491
Eosinophiles, %	-1,50	**+0,91**	-0,10	,020	,944	,8	,448	,505
Popovych's Strain Index-2, points	-0,163	**-0,004**	-0,032	,034	,546	11,6	,0002	,346
Micr Count vs St. aur., Bact/Phag	+0,6	**+3,8**	+0,35	,030	,630	8,2	,002	,292
$CD4^+$ T-helper Lymphocytes, %	+0,3	**+1,3**	+1,2	,022	,867	2,1	,136	,489
Killing Index vs Staph. aureus, %	+3,0	**-5,7**	+6,2	,041	,453	16,9	10^{-4}	,107
Killing Index vs E. coli, %	+5,5	**-5,9**	+7,7	,022	,845	2,6	,095	,264
Phagocytose Index vs E. coli, %	+0,22	**-1,12**	+0,01	,035	,537	12,1	,0002	,223
Variables currently not in the model Df for all F-tests: 2,27	**Cluster No.2 (10)**	**Cluster No. 3 (7)**	**Cluster No.1 (34)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Immunocytogram H	**+0,018**	-0,002	0,000	,018	,971	,40	,674	,493
Leukocytogram H	**-0,026**	-0,001	**+0,005**	,019	,995	,07	,935	,370
HRV H	**-0,012**	+0,011	**+0,030**	,018	,980	,28	,760	,570

Table 2. Summary of Stepwise Analysis for Changes in Variables of Entropy and Immunity in Clusters. The variables are ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
C3H	25,4	10^{-6}	,486	25,4	10^{-6}
O1H	14,8	10^{-5}	,298	19,5	10^{-6}
T3H	3,8	,030	,256	15,0	10^{-6}
Immune Status Index-11	4,0	,025	,217	12,9	10^{-6}
Eosinophiles, %	3,0	,058	,191	11,3	10^{-6}
F8H	2,9	,067	,168	10,3	10^{-6}
T4H	3,7	,033	,143	9,8	10^{-6}
Popovych's Strain Index-2, points	2,0	,151	,131	9,1	10^{-6}
Stub Neutrophils, %	2,4	,104	,117	8,6	10^{-6}
Killing Index vs Staph. aureus, %	2,5	,094	,103	8,2	10^{-6}
P3H	3,1	,058	,089	8,1	10^{-6}
F7H	2,5	,095	,078	7,9	10^{-6}
Phagocytose Index vs E. coli, %	2,0	,152	,071	7,7	10^{-6}
T6H	2,9	,067	,060	7,7	10^{-6}
Phagocytose Ind vs Staph. aur., %	2,7	,085	,052	7,7	10^{-6}
$CD4^+$ T-helper Lymphocytes, %	2,2	,130	,046	7,5	10^{-6}
$CD3^+$ T active Lymphocytes, %	2,0	,153	,041	7,4	10^{-6}
O2H	3,4	,047	,034	7,7	10^{-6}
Micr Count vs St. aur., Bact/Phag	3,8	,035	,027	8,0	10^{-6}
Leukocytes, 10^9/L	3,2	,056	,022	8,3	10^{-6}
Killing Index vs E. coli, %	2,6	,095	,019	8,4	10^{-6}

Next, the 21-dimensional space of discriminant variables transforms into 2-dimensional space of canonical roots. The canonical correlation coefficient is for Root 1 0,945 (Wilks' Λ=0,019; $\chi^2_{(42)}$=151; $p<10^{-6}$) and for Root 2 0,909 (Wilks' Λ=0,174; $\chi^2_{(20)}$=66; $p=10^{-6}$). The major root contains 63,8% of discriminative opportunities and the minor is 36,2%.

Table 3 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 (Fig. 2).

Table 3. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
C3H	,047	-1,175	,440	-10,95
O1H	-,714	,644	-4,569	4,120
T3H	,474	-,831	2,777	-4,868
Immune Status Index-11	-2,179	2,045	-3,624	3,401
Eosinophiles, %	-,243	-,264	-,137	-,149
F8H	1,468	,825	5,642	3,170
T4H	-,649	-,286	-3,920	-1,726
Popovych's Strain Index-2, points	1,209	-,080	3,945	-,259
Stub Neutrophils, %	2,315	-,670	1,598	-,463
Killing Index vs Staph. aureus, %	2,223	-,908	,217	-,089
P3H	-1,175	,304	-10,81	2,794
F7H	-1,317	,437	-5,715	1,897
Phagocytose Index vs E. coli, %	-1,429	,551	-,854	,329
T6H	1,038	-1,162	5,353	-5,993
Phagocytose Ind vs Staph. aur., %	,624	,275	,553	,244
$CD4^+$ T-helper Lymphocytes, %	-,337	-,454	-,067	-,090
$CD3^+$ T active Lymphocytes, %	,801	-,763	,156	-,148
O2H	-1,165	,648	-7,457	4,145
Micr Count vs St. aur., Bact/Phag	1,019	-,640	,113	-,071
Leukocytes, 10^9/L	,873	-,725	,762	-,633
Killing Index vs E. coli, %	,806	-,077	,060	-,006
	Constants		,208	-,911
	Eigenvalues		8,334	4,736
	Cum. Prop		,638	1,000

Table 4 shows the correlation coefficients of entropy and immunity changes (discriminant variables) with canonical discriminant roots, the cluster centroids of both roots, and the normalized entropy and immunity change values of the discriminant variables, as well as not included in the discriminant model. The reason for the last step is our experience that not getting a variable into the model does not always indicate a lack of recognition ability, but may be a consequence of redundancy (duplication) of information.

Table 4. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of changes in Variables for Clusters

Change in Variables	Correlations Variables-Roots		II (10)	III (7)	I (34)
Root 1 (63,8%)	R1	R2	**-4,43**	**-3,22**	**+1,97**
O1H	**-,292**	,076	**+1,38**	**+0,60**	**-0,56**
P3H	**-,239**	-,090	+0,83	**+1,16**	**-0,46**
O2H	**-,220**	,111	**+1,06**	**+0,12**	**-0,53**
F7H	**-,212**	,016	**+1,64**	**+1,10**	**-0,59**
T4H	**-,186**	-,075	+0,32	**+0,78**	**-1,27**
T6H	**-,178**	,037	**+1,16**	**+0,52**	**-0,61**
F8H	**-,139**	,153	**+1,29**	**-0,68**	**-0,78**
Phagocytose Ind vs Staph. aureus	**-,019**	-,005	**+0,13**	+0,13	**+0,06**
Fp2H	currently not in model		**+1,51**	**+0,50**	**-1,10**
T5H	currently not in model		**+1,42**	**+1,15**	**-0,59**
Fp1H	currently not in model		**+1,41**	**+1,17**	**-0,62**
P4H	currently not in model		**+0,66**	+0,70	**-0,52**
Immunocytogram H	currently not in model		**+0,31**	-0,03	**-0,01**
Immune Status Index-11	**,100**	,088	**+0,11**	-0,16	**+0,41**
Leukocytes	**,072**	,023	**-0,66**	**-0,85**	**+0,26**
Stub Neutrophils	**,051**	,006	**-0,17**	**-0,12**	**+0,56**
CD3⁺ T active Lymphocytes	**,033**	-,014	**-0,42**	**+0,03**	**+0,14**
HRV H	currently not in model		**-0,10**	**+0,11**	**+0,41**
Leukocytogram H	currently not in model		**-0,55**	**-0,01**	**+0,10**
Root 2 (36,2%)	R1	R2	+2,67	**-4,70**	+0,18
C3H	-,221	**-,371**	-0,13	**+2,68**	-0,89
T3H	-,125	**-,188**	-0,53	**+1,51**	-1,24
C4H	currently not in model		+0,09	**+2,82**	-0,91
F4H	currently not in model		+0,49	**+2,21**	-0,77
F3H	currently not in model		-0,15	**+1,74**	-0,57
Eosinophiles	,056	**-,175**	-1,72	**+1,04**	-0,11
Popovych's Strain Index-2	,110	**-,135**	-4,06	**-0,11**	-0,79
Microbial Count vs Staph. aureus	-,024	**-,051**	+0,06	**+0,38**	+0,04
CD4⁺ T-helper Lymphocytes	,018	**-,024**	+0,09	**+0,40**	+0,36
Immunoglobulins M	currently not in model		+0,05	**+0,58**	-0,10
CD22⁺ B Lymphocytes	currently not in model		+0,60	**+1,02**	+0,11
Killing Index vs Staph. aureus	,098	**,131**	+0,36	**-0,68**	+0,73
Killing Index vs E. coli	,075	**,127**	+0,57	**-0,61**	+0,80
Phagocytose Index vs E. coli	,024	**,113**	+0,19	**-0,95**	+0,01
Monocytes	currently not in model		-0,64	**-1,73**	+0,28
Bactericidity vs E. coli	currently not in model		+0,39	**-1,26**	+1,67
Bactericidity vs Staph. aureus	currently not in model		+0,25	**-0,98**	+1,64
Immunoglobulins A	currently not in model		-0,55	**-0,80**	+0,03
0-Lymphocytes	currently not in model		-0,40	**-0,75**	-0,15
Popovych's Adaptation Index-2	currently not in model		+0,58	**-0,14**	+0,62

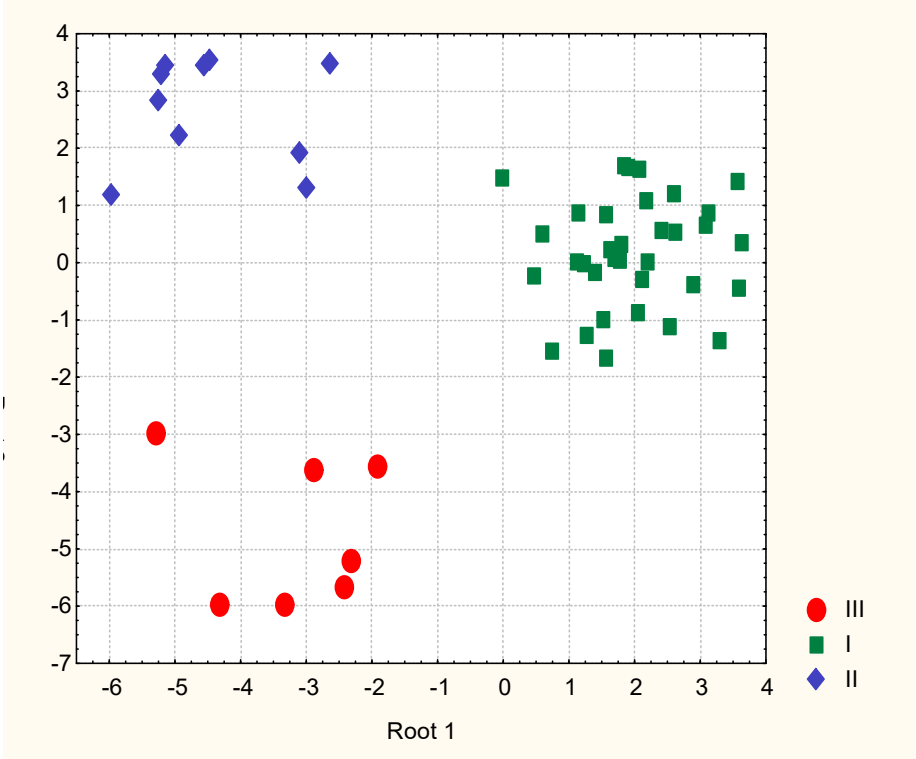

Fig. 2. Scatterplot of individual values of the first and second roots in which condensed information about of the changes in EEG Entropy and Immunity of the members of the three clusters

The localization of the members of the **first** cluster along the first root axis (Figs. 2 and 3) in the extreme right zone reflects decrease in entropy of EEG loci as well as minimal increase in phagocytose activity vs Staph. aureus, i.e. in variables which are related to the root **negatively,** while maximal increase in immune parameters which are related to the root **positively** (Table 4). The members of the other two clusters occupy extreme left position and their projections on the axis are mixed. Nevertheless, more left shift of the centroid of the second cluster results, as a rule, in a larger entropy increase.

Instead, along the second root axis (Figs. 2 and 4), members of these clusters are clearly deliminated due to the extremely lower position of the members of the **third** cluster, which reflects a significant increase in the entropy of the EEG loci as well as the immune parameters associated with the root **negatively**, combined with a decrease in the immune parameters related to the root **positively**.

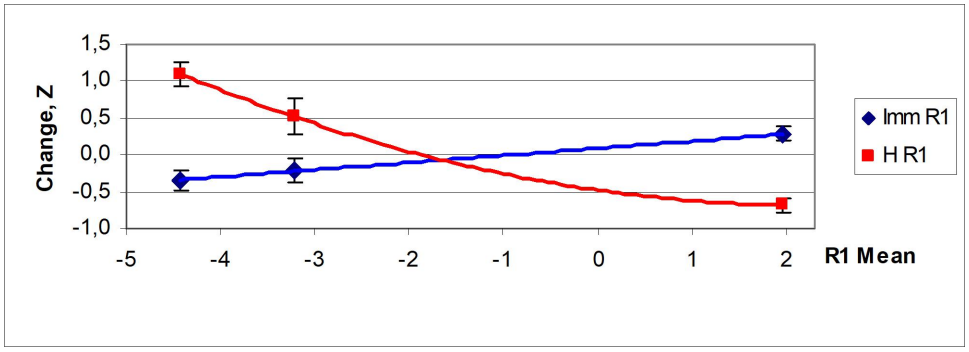

Fig. 3. Patterns of changes in EEG entropy and immunity parameters, the information of which is condensed in the first root

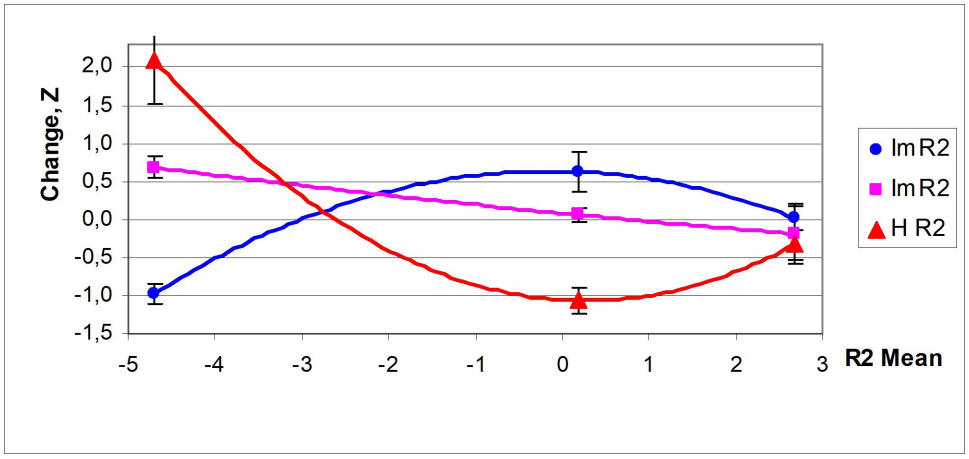

Fig. 4. Patterns of changes in EEG entropy and immunity parameters, the information of which is condensed in the second root

In general, all three clusters on the planes of the roots are clearly delineated, which is documented by calculating the Mahalanobis distances (Table 5).

Table 5. Squared Mahalanobis Distances between Clusters and F-values (for all $p<10^{-5}$)

	III	I	II
III	0	54	59
I	**7,6**	0	50
II	**5,9**	**9,8**	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 6). We can retrospectively recognize members of all clusters unmistakably (Table 7).

Table 6. Coefficients and Constants for Classification Functions of Clusters

	III	I	II
Change in Variables	p=,137	p=,667	p=,196
C3H	37,73	-13,51	-43,56
O1H	3,000	-,555	38,91
T3H	7,153	-2,234	-32,10
Immune Status Index-11	2,169	-,004	31,63
Eosinophiles, %	1,292	-,143	,361
F8H	-33,07	11,68	-16,51
T4H	17,58	-11,18	9,596
Popovych's Strain Index-2, points	-12,71	6,476	-19,39
Stub Neutrophils, %	-3,813	2,210	-9,157
Killing Index vs Staph. aureus, %	-,441	,249	-1,356
P3H	30,18	-12,24	63,85
F7H	12,77	-7,594	33,66
Phagocytose Index vs E. coli, %	1,119	-1,701	4,581
T6H	5,829	4,302	-44,83
Phagocytose Ind vs Staph. aur., %	-2,504	1,554	-1,375
$CD4^+$ T-helper Lymphocytes, %	,580	-,207	-,004
$CD3^+$ T active Lymphocytes, %	,046	,129	-1,235
O2H	9,373	-9,038	48,95
Micr Count vs St. aur., Bact/Phag	-,124	,114	-,783
Leukocytes, 10^9/L	-,816	,044	-6,406
Killing Index vs E. coli, %	-,190	,093	-,304
Constants	-15,63	-3,122	-19,36

Table 7. Classification Matrix for Clusters
Rows: Observed classifications; Columns: Predicted classifications

	Percent	III	I	II
Clusters	Correct	p=,137	p=,667	p=,196
III	100	**7**	0	0
I	100	0	**34**	0
II	100	0	0	**10**
Total	100	7	34	10

At the final stage of the analysis, we created three patterns of relationships between induced by adaptogenic balneotherapy changes in the SPD entropy the individual loci of EEG on the one hand, and the immune parameters, the information of which is condensed in two canonical discriminatory roots, on the other hand (Fig. 5).

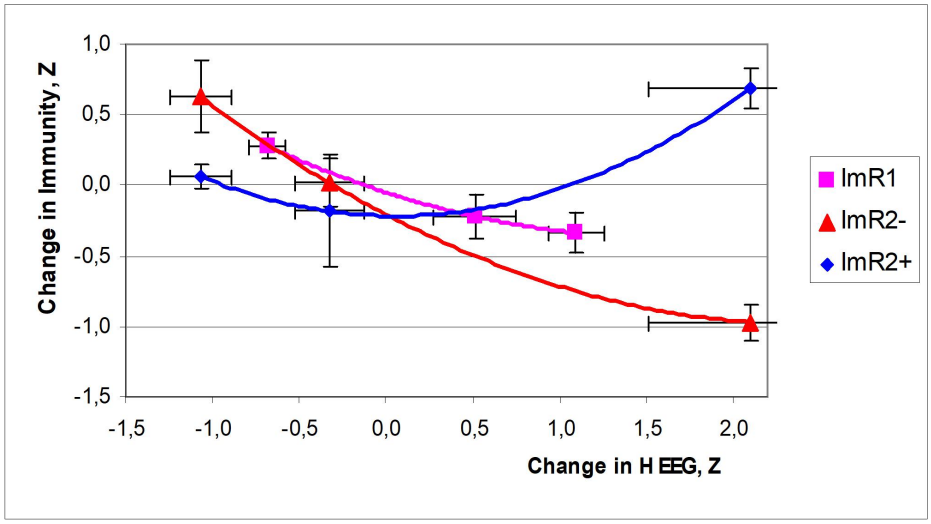

Fig. 5. Scatterplots of the correlations between changes in EEG entropy parameters and immunity parameters that are condensed in discriminative roots

As you can see, both inverse patterns are quite clear, but direct coupling occurs only as part of the increase in entropy, while its decrease is accompanied by the absence of changes in immune parameters.

Our data fits into the KJ Tracey's [38] scheme of immunological homunculus by which the neural structures that are projected onto definite loci responsible for certain immune functions, that is the immune compartment cytokines release (F3 and/or F4), activation of memory B cells (Fp1 and/or Fp2), dendritic cells maturation (T3 and/T4), regulation of T cells (T5 and/or T6), clonal expansion (P3 and/or P4) and late cytokine release (P? or O?).

We consider it appropriate to hypothesize that the immunomodulatory action of entropy of nerve structures is realized due to their effect on the tone of the vagus nerves, whose immunotropic effects are well documented [5,6,17,37,39]. In support of our hypothesis, we present the following provisions.

It is believed that a hippocampus is projected at the C3 and C4 loci, and the T3 and T4 loci reflect the activity of the amygdala [34]. The frontal loci record the activity of anterior cingulate [4] as well as orbito-frontal cortex. It is shown that the cortical thickness of an area within these regions positively correlated with two HRV-markers of parasympathetic activity both HF [16,41] and RMSSD [42]. It is shown significantly positive correlations between HFnu and Fz-θ, FCz-θ and Cz-θ [36]. Previously we [31,32] also found correlations between HFnu and F4-θ and P4-θ, between HF relative and Fp1-θ and P4-θ also between RMSSD and P4-θ. Prinsloo GE et al. [33] found that less pronounced changes in HRV, due to work-related stress, accompanied by higher relative SPD Fz-θ, Pz-θ and Cz-θ, lower fronto-central relative β power and higher θ/β ratio. It is also perfectly consistent with our [31,32] data on a negative correlation LFnu, LFr and LF/HF with F4-θ, P4-θ, F7-θ, F8-θ and positive - with F7-β and F8-β - on the one hand, and a positive correlation HFr with Fp1-θ and P4-θ and negative - with P4-β - on the other side.

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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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