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INTERPERSONAL DIFFERENCES BETWEEN OF THE ENTROPIES OF EEG, HRV, IMMUNOCYTOGRAM AND LEUKOCYTOGRAM

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Background. Previously, we have shown that in humans entropy of the normalized parameters of the HRV and SPD of loci of EEG significantly correlate with the entropy of Leukocytogram and Immunocytogram as well as parameters of immunity, which testifies to their modulating regulatory effects. This article is a direct extension of the previous one. Its purpose is the distribution of the observed contingent into groups that are homogeneous in terms of entropy of the HRV, EEG as well as Leukocytogram and Immunocytogram. **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"). In blood we determined relative content of components (RCC) of Immunocytogram (ICG) (T helper, T cytolytic, B and NK lymphocytes) and Leukocytogram (LCG) (Eosinophils, Stub and Segmentonuclear Neutrophils, Lymphocytes and Monocytes). Than we calculated for each locus of EEG and HRV as well as for ICG and LCG the Entropy (h) of normalized spectral power density (SPD) or RCC using Shannon's formula. **Results.** The method of cluster analysis is revealed that in members of the major cluster (60%), the entropy of EEG, HRV, ICG and LCG varies within the normal range ($-0,5\sigma \div +0,5\sigma$). The members of the next largest cluster (23%)

are characterized by a moderately increased entropy of the SPD of EEG in conjunction with the normal entropy of the ICG and the moderately reduced entropy of HRV and LCG. The members of the third cluster (9%) noted a significantly lower entropy (negentropy) of the SPD in loci F3, F4, T3 and C4; in addition, there is a moderate decrease in the entropy of the LCG. Instead, members of the last cluster (8) noted the negentropy of SPD in paired loci Fp1 and Fp2, T5 and T6, T3 and T4, F7 and F8 as well as O1 and O2; in addition, there is a moderate decrease in entropy of the ICG. The entropy of other EEG locus as well as of HRV and LCG is within the normal range. **Conclusion.** The entropy of HRV and SPD of loci of EEG as well as ICG and LCG is characterized by a large variation, which corresponds to the known wide variance of parameters of the ICG and LCG, which are subordinate to the regulatory influences of the central and autonomic nervous system.

Key words: EEG; HRV; Leukocytogram; Immunocytogram; Entropy; Clusters; Women and Man.

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 [9]. This article is a direct extension of the previous one. Its purpose is the distribution of the observed contingent into groups that are homogeneous in terms of entropy of the HRV, EEG as well as Leukocytogram and Immunocytogram.

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"). In blood we determined relative content of components (RCC) of Immunocytogram (ICG) (T helper, T cytolytic, B and NK lymphocytes) and Leukocytogram (LCG) (Eosinophils, Stab and Segmentonuclear Neutrophils, Lymphocytes and Monocytes). Then we calculated for each locus of EEG and HRV as well as for ICG and LCG the Entropy (h) of normalized spectral power density (SPD) or RCC using Shannon's formula. See the previous article for details [9].

The reference values of the Entropy parameters are taken from the database of Truskavets' Scientific School.

RESULTS AND DISCUSSION

To achieve the declared goal, a Cluster analysis was applied. Use of Cluster analysis makes possible the simultaneous consideration of all the signs. Considering the totality of characteristics of persons undertaken in their relationship and conditionality of some of these (derivatives) other (main determinants) allows as to make a natural classification that reflects the nature of things, their essence. It is believed that knowledge of the essence of the object is to identify those of its quality properties that actually define the object, distinguish it from other [1,7].

Clustering cohort of persons is realized by iterative k-means method. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points [1,7].

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

$$\eta^2 = S_b^2 / (S_b^2 + S_w^2),$$

$$R = \eta,$$

$$F = [S_b^2(n-k)] / [S_w^2(k-1)], \text{ where}$$

S_b^2 is Between Variance;

S_w^2 is Within Variance;

n is number of persons (102);

k is number of groups-clusters (4).

In our case, the maximum coefficient η^2 , is the largest contribution to the division into clusters, stated for the Entropy of the SPD of EEG in loci T6 and Fp2, while the minimum contribution between them is given by the Entropy of the SPD of the EEG in loci P4 and T3. Even smaller but statistically significant contributions to clustering are given by the Entropies of LCG and ICG, while the contribution of the Entropy of HRV is negligible (Table 1).

Table 1. Analysis of Variance of Entropy of SPD of EEG as well as HRV, Leukocytogram and Immunocytogram

Variables	Between SS	Within SS	η^2	R	F	signif. p
T6H	1,662	,923	0,643	0,802	49,2	10^{-6}
Fp2H	1,167	,652	0,642	0,801	49,0	10^{-6}
F3H	1,249	,893	0,583	0,764	38,2	10^{-6}
T5H	1,455	1,365	0,516	0,718	29,2	10^{-6}
O1H	1,228	1,295	0,487	0,698	25,9	10^{-6}
F4H	1,143	1,208	0,486	0,697	25,9	10^{-6}
F8H	1,961	2,125	0,480	0,693	25,2	10^{-6}
F7H	1,636	1,924	0,460	0,678	23,2	10^{-6}
O2H	1,143	1,385	0,452	0,672	22,6	10^{-6}
Fp1H	,752	1,207	0,384	0,620	17,0	10^{-6}
C3H	,453	,811	0,358	0,599	15,3	10^{-6}
C4H	,526	,976	0,350	0,592	14,7	10^{-6}
T4H	,576	1,127	0,338	0,582	14,0	10^{-6}
P3H	,453	,925	0,329	0,573	13,4	10^{-6}
P4H	,441	1,040	0,298	0,546	11,6	10^{-5}
T3H	,620	1,523	0,289	0,538	11,1	10^{-5}
LCGH	,021	,172	0,109	0,330	3,3	,024
ICGH	,024	,236	0,092	0,304	2,8	,045
HRVH	,028	1,235	0,022	0,149	0,6	,602

Actual mean values of entropy of SPD in loci of EEG as well as of HRV, LCG and ICG in members of various clusters are rendered in Fig. 1.

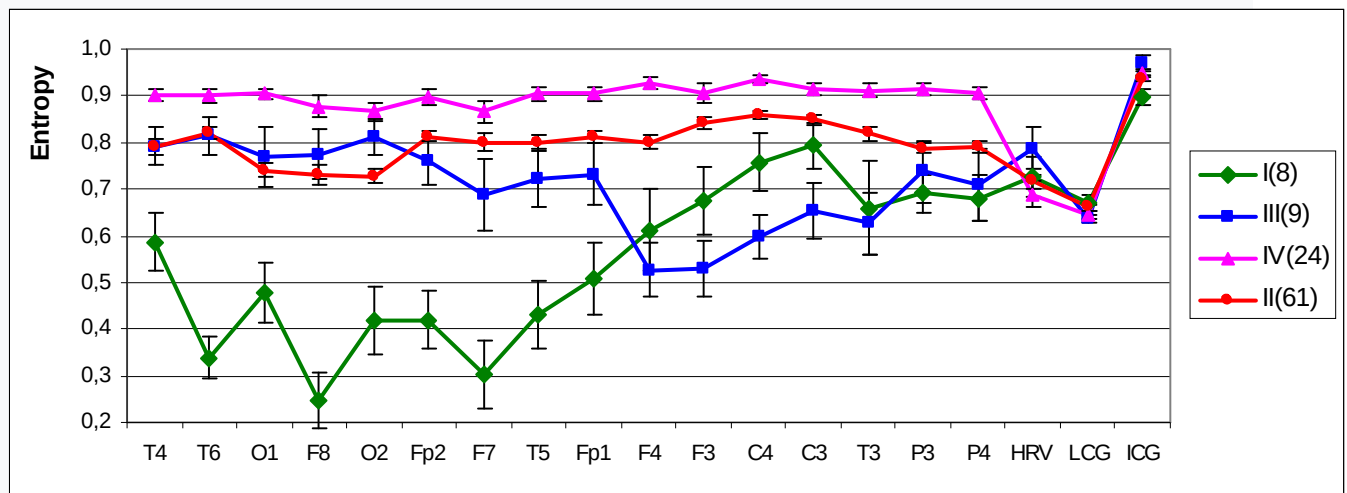


Fig. 1. Actual mean values (M±SE) of entropy of SPD in loci of EEG as well as of HRV, LCG and ICG in members of various clusters

However, as demonstrated in numerous studies by the Truskavets' Scientific School, the expression of parameters in the format of Z-scores is more adequate in relation to physiological significance, that is, taking into account their variability in the norm [2,3,5,6,8].

This approach is implemented and visualized in Figure 2.

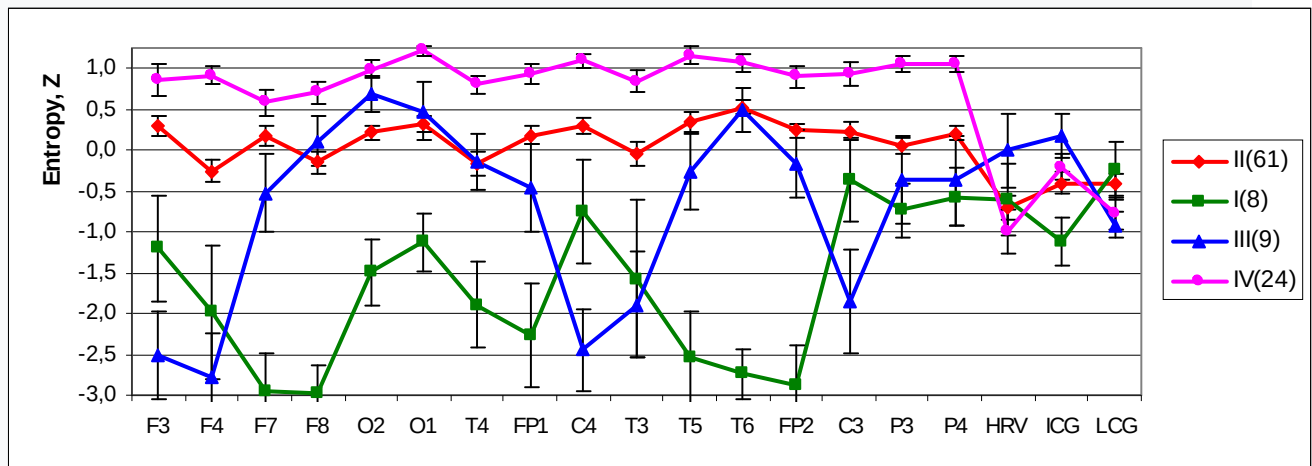


Fig. 2. Z-scores ($M \pm SE$) of entropy of SPD in loci of EEG as well as of HRV, LCG and ICG in members of various clusters

It turned out that in members of the major **second** cluster, the entropy of EEG, HRV, ICG and LCG varies within the normal range ($-0,5\sigma \div +0,5\sigma$). The members of the next largest **fourth** cluster are characterized by a moderately increased entropy of the SPD of EEG in conjunction with the normal entropy of the ICG and the moderately reduced entropy of HRV and LCG. The most stringent were the members of two minor clusters. In particular, members of the **third** cluster noted a significantly lower entropy (negentropy) of the SPD in paired loci F3 and F4 responsible, as implied by KJ Tracey [11], for the immune compartment cytokine release, T3 (but not T4) responsible for the maturation of dendritic cells, as wells as C4, responsible, already according to our assumption [9], for increasing the intensity of phagocytosis by neutrophils of Gram-positive and Gram-negative microbes and C3 responsible for increasing the content in the blood of IgM and total lymphocytes as well as reducing the content of IgA and segmentonuclear neutrophils. In addition, there is a moderate decrease in the entropy of the LCG. The entropy of other EEG loci as well as of HRV and ICG is within the normal range.

Instead, members of the **first** cluster noted the negentropy of SPD in paired loci Fp1 and Fp2, responsible according to KJ Tracey [11], for the activation of memory T cells, T5 and T6 responsible for T cell regulation, T3 and T4, responsible, repeat, for maturation of dendritic cells, as wells as less pronounced than in the third cluster, the decrease in entropy in loci F3 and F4 responsible for the immune compartment cytokine release. In addition, there is a moderate decrease in the entropy in loci O1 and O2 responsible for the strain of the leukocyteogram as well as the inhibition of completeness of phagocytosis of Staph. aureus, but not E. coli [9]. However, the responsibility for immunomodulation of the structures that are projected on the F7 and F8 locus with maximum negentropy remains unclear. In addition, there is a moderate decrease in entropy of the ICG. The entropy of other EEG locus as well as of HRV and LCG is within the normal range.

The average normalized values of the entropy of the SPD of the EEG, HRV, immunocytogram and leukocyteogram are shown in figure 3. As we see, the characteristic features of the image of the members of the first cluster are expressed negentropy of the EEG in general,

moderate negentropy of the Immunocytogram, lower boundary level of the entropy of the HRV, and the normal level of entropy of the Leukocytogram. Members of the third cluster are characterized by moderate negentropy of EEG and Leukocytogram in combination with normal levels of entropy of the HRV and Immunocytogram. Instead, the members of the fourth cluster are characterized by increased entropy of the EEG, coupled with a reduced entropy of HRV and Leukocytogram at normal levels of entropy of the Immunocytogram. However, for the vast majority of people who make up the second cluster, the normal entropy of all analyzed information systems is typical.

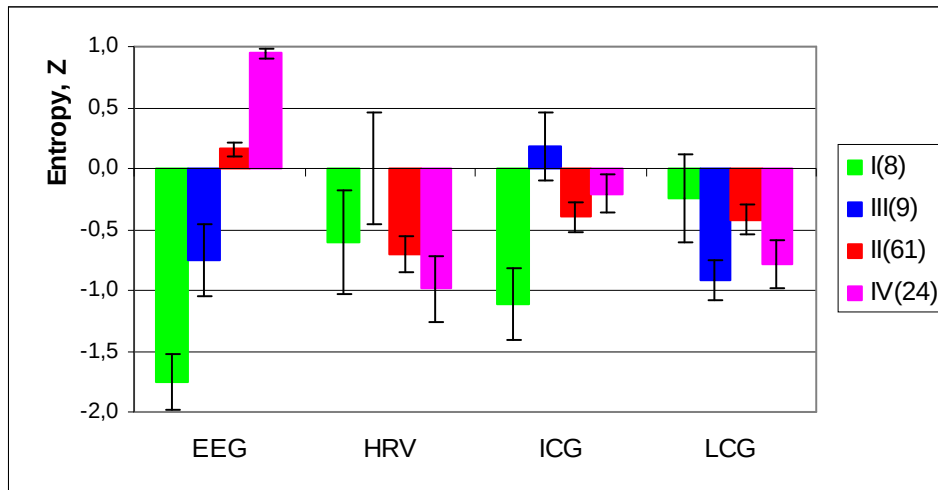


Fig. 3. Normalized entropy of SPD of loci of EEG, HRV, Immunocytogram and Leukocytogram

In order to identify exactly those parameters (variables) whose constellation is characteristic for each cluster, the available informational field was subjected to discriminant analysis by the method of forward stepwise [4]. To include in the model (Table 2), the program has selected only 15 variables, while the other 4 were outside the discriminant model.

Table 2. Discriminant Function Analysis Summary for Entropies

Step 15, N of vars in model: 15; Grouping: 4 grps

Wilks' Lambda: 0,0376; approx. $F_{(45)}=11,2$; $p < 10^{-6}$

Variables currently in the model	IV (24)	II (61)	III (9)	I (8)	Wilks' Λ	Partial Λ	F-remove (3,8)	p-level	Tolerance	Norm level (88)	Cv
T6H	0,900	0,820	0,814	0,338	,042	,890	3,45	,020	,681	0,742	0,199
Fp2H	0,896	0,813	0,760	0,420	,040	,934	1,97	,125	,689	0,782	0,161
F8H	0,877	0,731	0,775	0,246	,042	,898	3,20	,028	,707	0,757	0,226
F7H	0,865	0,800	0,688	0,301	,040	,937	1,89	,138	,706	0,772	0,207
T5H	0,904	0,799	0,723	0,432	,039	,964	1,05	,374	,682	0,756	0,169
O2H	0,865	0,728	0,812	0,420	,043	,869	4,22	,008	,657	0,688	0,261
O1H	0,904	0,741	0,769	0,479	,041	,924	2,30	,083	,617	0,682	0,266
F4H	0,927	0,800	0,527	0,613	,040	,949	1,49	,223	,594	0,828	0,131
T4H	0,903	0,789	0,792	0,585	,045	,844	5,19	,002	,631	0,809	0,146
P3H	0,913	0,788	0,738	0,690	,042	,888	3,52	,018	,559	0,782	0,159
T3H	0,911	0,819	0,627	0,659	,042	,899	3,16	,029	,501	0,823	0,126
ICGH	0,948	0,937	0,970	0,897	,039	,952	1,41	,245	,890	0,960	0,059
C4H	0,934	0,858	0,597	0,758	,047	,808	6,66	,0004	,622	0,830	0,115
F3H	0,905	0,844	0,530	0,676	,048	,782	7,81	,0001	,586	0,810	0,137
HRVH	0,689	0,717	0,787	0,727	,041	,925	2,27	,086	,841	0,788	0,127
Variables currently not in the model	IV (24)	II (61)	III (9)	I (8)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerance		
Fp1H	0,905	0,812	0,732	0,508	,036	,969	,90	,446	,498	0,781	0,157
P4H	0,908	0,790	0,709	0,681	,037	,991	,25	,864	,465	0,761	0,184
C3H	0,915	0,848	0,653	0,793	,037	,985	,43	,734	,537	0,827	0,114
LCGH	0,644	0,661	0,637	0,669	,037	,993	,19	,905	,755	0,681	0,070

Table 3. Summary of Stepwise Analysis for Entropies. The variables are ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
T6H	69,8	10^{-6}	,319	70	10^{-6}
C4H	32,7	10^{-6}	,159	49	10^{-6}
F3H	10,0	10^{-5}	,121	36	10^{-6}
O2H	7,4	10^{-3}	,098	29	10^{-6}
T4H	6,5	10^{-3}	,081	26	10^{-6}
P3H	4,0	,010	,072	23	10^{-6}
F8H	5,1	,003	,062	21	10^{-6}
T3H	2,9	,040	,056	19	10^{-6}
F7H	3,0	,036	,051	17	10^{-6}
HRVH	1,7	,177	,048	16	10^{-6}
ICGH	1,6	,206	,046	15	10^{-6}
O1H	1,5	,230	,044	13	10^{-6}
Fp2H	2,0	,123	,041	13	10^{-6}
F4H	1,4	,249	,039	12	10^{-6}
T5H	1,1	,374	,038	11	10^{-6}

Next, the 15-dimensional space of **discriminant variables** transforms into 3-dimensional space of a **canonical discriminant functions** (canonical roots), which are a linear combination of discriminant variables. The discriminating (differentiating) ability of the root characterizes the canonical correlation coefficient (r^*) as a measure of connection, the degree of dependence between groups (clusters) and a discriminant function. It is for Root 1 0,926 (Wilks' $\Lambda=0,038$; $\chi^2_{(45)}=300$; $p<10^{-6}$), for Root 2 0,800 (Wilks' $\Lambda=0,265$; $\chi^2_{(28)}=121$; $p<10^{-6}$), for Root 3 0,512 (Wilks' $\Lambda=0,738$; $\chi^2_{(13)}=28$; $p=0,0097$). The first root contains 73,9% of discriminative opportunities, the second is 21,8% and the third only 4,3%, therefore, will continue to be ignored.

Table 4 presents raw (actual) and standardized (normalized) coefficients for discriminant variables. The raw coefficient gives information on the **absolute** contribution of this variable to the value of the discriminative function, whereas standardized coefficients represent the **relative** contribution of a variable independent of the unit of measurement. They make it possible to identify those variables that make the largest contribution to the discriminatory function value.

The same is the **full structural coefficients**, that is, the coefficients of correlation between the discriminant root and variables. The structural coefficient shows how closely variable and discriminant functions are related, that is, what is the fate of information about the discriminant function (root) contained in this variable.

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. 4).

Table 4. Standardized, Structural and Raw Coefficients and Constants for Entropic Variables

Coefficients Variables	Standardized			Structural			Raw		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
T6H	-,322	-,185	-,437	-,572	-,247	-,350	-3,332	-1,913	-4,513
Fp2H	-,135	-,245	-,398	-,503	-,084	-,229	-1,413	-2,570	-4,176
F8H	-,387	-,064	,231	-,417	-,196	,021	-2,612	-,434	1,564
F7H	-,293	,007	-,244	-,404	-,028	-,365	-2,059	,051	-1,715
T5H	-,131	,207	-,205	-,393	,002	-,068	-1,069	1,686	-1,673
O2H	-,339	-,394	,067	-,370	-,230	,251	-2,928	-3,397	,578
O1H	,019	,164	,635	-,353	-,083	,374	,162	1,370	5,313
F4H	-,137	,262	,309	-,320	,480	,134	-1,200	2,292	2,703
T4H	-,352	-,355	,481	-,286	-,042	,243	-3,168	-3,195	4,327
P3H	-,401	,035	,483	-,238	,142	,450	-4,047	,357	4,871
T3H	,485	-,008	,058	-,221	,284	,063	3,729	-,059	,445
ICGH	-,166	-,187	,176	-,085	-,163	,044	-3,128	-3,527	3,328
C4H	-,063	,671	-,250	-,266	,635	,044	-,744	7,897	-2,937
F3H	-,563	,388	-,119	-,249	,518	-,153	-4,919	3,389	-1,037
HRVH	-,177	-,312	-,011	,037	-,136	-,026	-1,540	-2,710	-,096
Eigenvalues	6,055	1,784	,355	Constants			22,57	,364	-5,741
Cum. Prop.	,739	,957	1,000	Discriminant Properties, %			73,9	21,8	4,3

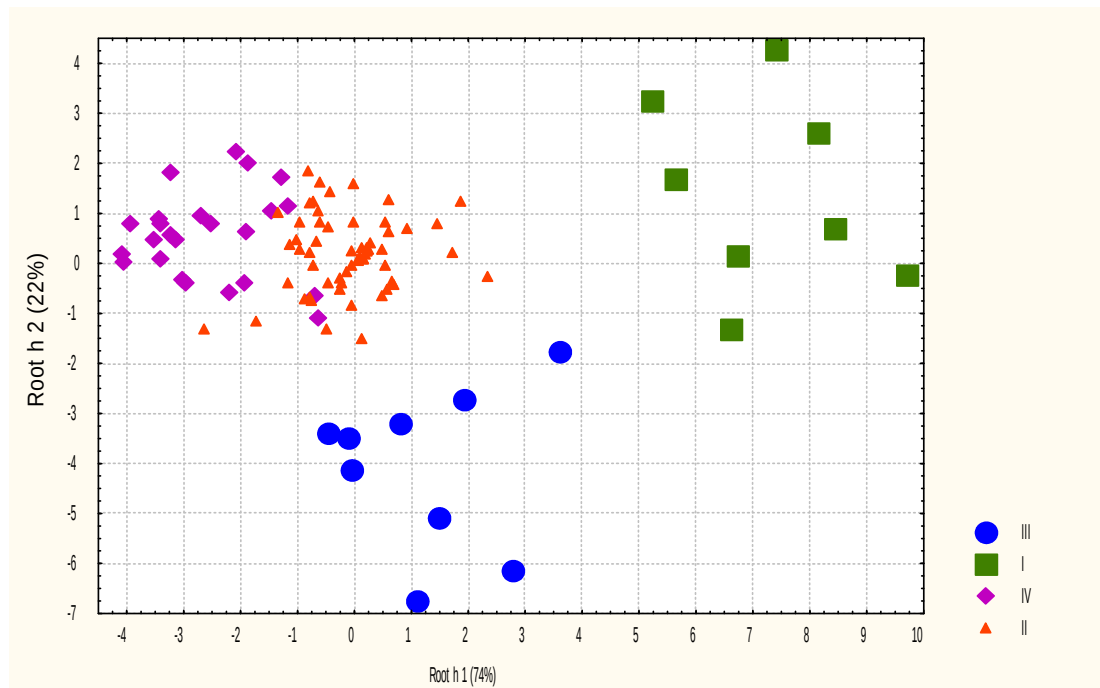


Fig. 4. Individual values of the two roots of the entropy of the members of the four clusters

The localization of the members of the first cluster along the first root axis in the extreme right (positive) zone (centroide: +7,24) reflects the expressed integral negentropy of SPD of 11 loci of EEG as well as ICG, which are related to the root **negatively** (Tables 2 and 4). Instead, the

fourth cluster has an extreme left (negative) zone (centroide: -2,58), which reflects the increased integral entropy of these parameters. The members of the other two clusters occupy an intermediate position and their projections on the axis are mixed. Nevertheless, the positive value of the centroid of the third cluster (+1,23) reflects the lower boundary level of the integral entropy of its parameters, and the magnitude of the centroid of the second cluster (-0,12), which is close to zero, characterizes the variation of the entropy of its parameters around zero. Instead, along the second root axis, members of the third cluster (centroide: -4,08) are clearly separated from the members of both the second and two other clusters whose projections are shifted to the axis (centroids: +0,20; +0,55 and +1, 39 for II, IV and I cluster respectively). This disposition reflects the minimum values for the entropy of SPD in loci C4 and F3, which are related to the root **positively**, while the maximum entropy of HRV which is related to the root **negatively** (Tables 2 and 4).

In general, all four entropy clusters on the plane of the first two roots, which together contain 95,7% of the discriminant information, are quite clearly delineated, which is documented by calculating the Mahalanobis distances (Table 5).

Table 5. Squared Mahalanobis Distances between Clusters of Entropy and F-values (df=15,8; for all pairs $p < 10^{-6}$)

Clusters	III	I	IV	II
III	0	69	38	22
I	14,7	0	101	59
IV	12,8	31,0	0	8
II	8,7	21,2	7,8	0

The same discriminan parameters can be used to identify (classify) the belonging of one or another rat to an intact group or subject to water-salt loading. This purpose of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 6). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. The coefficients of the classifying functions are not standardized, therefore they are not interpreted. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant.

Table 6. Coefficients and Constants for Classification Functions of Clusters of Entropy

Clusters	III	I	IV	II
Variables	p=,088	p=,078	p=,235	p=,598
T6H	26,05	-6,14	27,87	26,11
C4H	9,42	47,02	47,48	46,63
F3H	130,5	119,1	164,4	152,4
O2H	69,63	33,67	65,33	58,56
T4H	126,7	91,80	125,9	113,7
P3H	95,94	75,42	115,2	98,86
F8H	67,86	50,39	76,51	68,22
T3H	-72,19	-49,94	-86,48	-77,83
F7H	24,45	11,70	31,76	28,86
HRVH	117,4	93,28	110,7	107,9
ICGH	421,7	384,8	418,8	408,0
O1H	24,04	34,52	32,14	25,26
Fp2H	21,35	-2,79	12,96	15,73
F4H	-6,27	,09	10,13	2,90
T5H	-0,85	1,31	10,27	9,19
Constants	-442,5	-325,5	-523,1	-455,6

In this case, we can retrospectively recognize members of third and first clusters **unmistakably**, the second cluster is classified with one bug, and only the fourth cluster with three errors. Overall classification accuracy is 96,1% (Table 7).

Table 7. Classification Matrix for Clusters of Entropy

Rows: Observed classifications; Columns: Predicted classifications

Clusters	Percent correct	III	I	II	IV
		p=,088	p=,078	p=,598	p=,235
III	100	9	0	0	0
I	100	0	8	0	0
II	98,4	0	0	60	1
IV	87,5	0	0	3	21
Total	96,1	9	8	63	22

The obtained results indicate that the levels of entropy of the HRV and of the SPD of the EEG loci as well as the ICG and LCG in the vast majority of the observed patients are within the normal range, however, take place both a moderate increase in entropy and a moderate and significant decrease in its level. Taking into account the previously established connections between the parameters of entropy and immunity [9], one can assume that each of the entropy clusters is characterized by a specific constellation of immunity parameters. Checking this hypothesis will be devoted to our next article.

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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