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Original Article

Effects of rehabilitation at the Truskavets' spa on physical working capacity and its neural, metabolic, and hemato-immune accompaniments

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Abstract:
Background. Ergometric **physical working capacity** (PWC) testing has a long tradition in occupational medicine for assessing whether a sufficiently high level of physical performance for coping with the daily work requirements is given. Physical performance and stress resistance of the body are considered one of the main objects of influence of adaptogens. The stress-limiting effect of bioactive Naftussya water known. Data on the influence of Naftussya on the muscular performance are ambiguous. We set ourselves the goal of comparing balneotherapy-induced individual changes in PWC with changes in some parameters of the nervous and immune systems, as well as erythron, hemostasis, and metabolism. **Material and methods.** The object of observation were 19 men and 3 women (age 26÷61 years, body weight 78÷100 kg) with urate urolithiasis and chronic pyelonephritis. The survey was conducted twice: on admission and after two weeks of rehabilitation at the Truskavets' Spa. Registered PWC_{150}, parameters of HRV, EEG, immunity, phagocytosis, erythron, hemostasis and metabolism. **Results**. The analysis of individual changes revealed that in 45,5% patients reduced fitness was completely normalized, however, in 54,5% patients, the normal level of fitness fell to the lower zone of the norm. Discriminant analysis revealed 5 EEG parameters, VLF band HRV as well as plasma alanine aminotransferase and bactericidal capacity of monocytes of blood, the changes of which are characteristic (classification accuracy 100%) for alternative variants of actotropic effects of balneotherapy. **Conclusion**. The alternative response of fitness to balneofactors occurs within the framework of the functional-metabolic continuum and the neuro-immune complex. A decrease in fitness to the lower normal zone is accompanied by the normalization of the reduced bactericidal capacity of blood monocytes, so we interpret this decrease as a "physiological payment" for it.
Keywords: **physical working capacity, HRV, EEG, immunity, phagocytosis, erythron, hemostasis, metabolism, Naftussya bioactive water, Truskavets' spa.**

Introduction

Ergometric **physical working capacity** (PWC) testing has a long tradition in occupational medicine for assessing whether a sufficiently high level of physical performance for coping with the daily work requirements is given (Sammito S et al., 2020; Steinhilber B et al., 2022). An imbalance between physical workload and physical work capacity related to aging workers has been suggested to result in chronic overload, increasing the risk of long-term health effects (De Zwart BC et al., 1995; Kenny GP et al., 2008). PWC can be tested maximally or submaximally, using performance indicators like VO_2max (Bugajska J et al., 2011) or the mechanical power (Farazdaghi GR & Wohlfart B, 2001; Wohlfart B & Farazdaghi GR, 2003). In the case of submaximal PWC testing measuring the mechanical power, the achieved power at a given heart rate serves as performance indicator. There are age- and sex-specific norm values (Stemper T, 1988) that can be used to judge whether differences or changes are within the normal range or can be considered significant.

We have previously demonstrated the role of innate muscular endurance in reactions to acute stress of neuro-endocrine-immune compex in rats (Fil VM et al., 2021; Zukow W et al., 2022). Physical performance and stress resistance of the body are considered one of the main objects of influence of adaptogens and are used for quantitative assessment of adaptogenic activity (Brekhman II, 1968; Panossian AG et al., 2021). The stress-limiting effect of bioactive Naftussya water as the main healing factor of Truskavets' spa is known (Popovych

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IL, 2011). Therefore, it is interesting to find out how the bioactive Naftussya water affects this important component of the body's general resistance. Data on the influence of balneotherapy at the Truskavets' spa on the muscular performance of patients (adults and children), assessed by veloergometric or step tests, are ambiguous (Ruzhylo SV et al., 2003; Popovych IL et al., 2005; Zukow W et al., 2020; Zukow W et al., 2021). Since the balneotherapy complex includes, in addition to drinking Naftussya water, ozokerite applications and mineral baths (Popovych IL et al., 2003), a controlled experiment was conducted on rats that received only Naftussya water for 3 weeks. But even under these conditions, the results of the test with swimming to exhaustion turned out to be ambiguous: in 33,3% of the animals, a significant increase in working capacity was noted, in another 44,4% - a moderate increase, while in 22,2% it decreased (Ruzhylo SV et al., 2003; Popovych IL et al., 2005). The authors explain such polyvariance of actotropic effects by the individual reactivity of the body. Based on the above, we set ourselves the goal of comparing balneotherapy-induced individual changes in PWC with changes in some parameters of the nervous and immune systems, as well as erythron, hemostasis, and metabolism.

Material and methods

Participants. Research with the participation of two authors (Popovych IL & Tserkovniuk RG) was conducted back in 1997 as part of the project "Chornobyl', Adaptive and Defensive Systems, Rehabilitation". The results are published in a number of articles and two monographs (Popovych IL et al., 2003; Kostyuk PG et al., 2006). However, due to certain circumstances of a technical, methodological and ethical nature, the results of electroencephalography and bicycle ergometry remained unanalysed. The object of observation were 19 men (age 26÷61 years, body weight 78÷100 kg) and 3 women (38, 40 and 47 years) with urate urolithiasis and chronic pyelonephritis who were exposed to pathogenic factors of the accident at the Chornobylian nuclear power plant during the liquidation of its consequences in 1986-87. According to the documents, the total effective radiation dose was 10÷25 cGy, which is most typical for this contingent (Romodanov AP, 1993; Popovych IL et al., 2003). The survey was conducted twice: on admission and after two weeks of rehabilitation at the Truskavets' Spa: bioactive water Naftussya by 3 mL/kg for 1 hour before meals three times a day; baths with mineral water (Cl^{-}-SO_4^{2-}-Na^{+}-Mg^{2+} containing salt concentration 25 g/L; t^0 36-37^{0}C during 8-10 min); therapeutic physical education (motion mode II).

Procedure / Test protocol / Skill test trial / Measure / Instruments.

For estimation of physical working capacity (PWC) a bicycle ergometer "Tunturi" (Finland) is used. The power of the first load was 0,5 W/kg at a pedaling frequency of 60-75 rpm. The power of the second load (after 3 min), according to the recommendations for a gentle version of the PWC test, taking into account the age of the subjects (Belotserkovskiy ZB, 1986), was selected so that the heart rate (HR) at the end of the load was close to that calculated by the formula: HR = (220 - Age)•0,87. This fully corresponded to the later recommendations for ergometer testing in occupational medicine (Trappe H-J & Löllgen H, 2000; Finger JD et al, 2013; Chatterjee M & Schmeißer G, 2017). Calculated submaximal PWC_{150} with the mechanical power in Watt per kilogram body weight (W/kg) as indicator of cardiorespiratory fitness (Finger JD et al, 2013). Systolic (Ps) and diastolic (Pd) blood pressure was measured by tonometer "Omron M4-I" (Netherlands) in a sitting position. Then we recorded electrocardiogram in II lead to assess the parameters of heart rate variability (HRV) (software and hardware complex "CardioLab+HRV" produced by "KhAI-MEDICA", Kharkiv, Ukraine). For further analysis the following parameters HRV were selected. Temporal parameters (Time Domain Methods): HR, the mode (Mo), 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 than 50 msec (pNN_{50}); triangular index (TNN). Spectral parameters (Frequency Domain Methods): absolute (msec^2) and relative (%) power spectral density (PSD) 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 ultralow-frequency (ULF, range 0,015÷0,003 Hz) (HRV, 1996). Baevskiy's parameters: the amplitude of mode (AMo) and variational sweep (MxDMn). The same parameters and Mode were also recorded for 2 minutes immediately after the end of the load (Baevskiy RM et al., 1984; 2001). The good old Kerdoe's Vegetative Index was also calculated (Kerdö I, 1966; Fajda OI et al., 2015). EEG recorded at rest during 25 sec a 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 earlobes. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%), absolute (μV^2/Hz) and relative (%) PSD of basic rhythms: 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.

In addition, we calculated for HRV and each locus of EEG the Entropy (h) of normalized PSD using Popovych's IL (Popadynets' et al., 2020) formulas based on classic Shannon's CE (1948) formula:

$$hEEG = - [PSD\alpha \cdot \log_2 PSD\alpha + PSD\beta \cdot \log_2 PSD\beta + PSD\theta \cdot \log_2 PSD\theta + PSD\delta \cdot \log_2 PSD\delta]/\log_2 4;$$

$$hHRV = - [PSDHF \cdot \log_2 PSDHF + PSDLF \cdot \log_2 PSDLF + PSDVLF \cdot \log_2 PSDVLF + PSDULF \cdot \log_2 PSDULF]/\log_2 4.$$

Since immunogenesis, hemostasis, erythron and non-specific resistance of the organism are closely interrelated (Kuznik BI et al., 1985; Drannyk GN, 1989; Popovych IL et al, 2003), their parameters were included in the battery of tests. The parameters of immunity were determined as described in the manual (Perederiy VG et al., 1995). About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by the phagocytosis index (PhI), the microbial count (MC) and the killing index (KI) for Staphylococcus aureus (ATCC N25423 F49) (Douglas SD & Quie PG, 1981; Bilas VR & Popovych IL, 2009; Popovych IL et al, 2003). On the basis of the registered partial parameters of phagocytosis, taking into account the content of neutrophils (N) or monocytes (M) in 1 L of blood, the integral parameter - the bactericidal capacity of neutrophils/monocytes - was calculated by the formula:

BCCN/M (10^9 Bact/L) = N/M (10^9/L)•PhI (%)•MC (Bacteria/Phagocyte)•KI (%)•10^{-4}.

The state of cellular immunity judged by the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant and theophylline-sensitive subpopulations (by the test of sensitivity of rosette formation to theophylline) as well as subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. Additional evaluated the transformation of T-lymphocytes into blasts under the influence of phytohemagglutinin (PhHA). The state of humoral immunity judged by the relative content of the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep, the concentration in serum circulating immune complexes and Immunoglobulins classes M, G, A. Among the parameters of hemostasis, the blood platelet content, kaolin-activated plasma recalcification time, prothrombin index, fibrinogen A and fibrinogen B plasma content, plasma tolerance to heparin were determined as described in the manual (Goryachkovskiy AM, 1998).

Finally, according to the protocol, routine hematological (hemoglobin, erythrocytes, reticulocytes, hematocrit, erythrocyte sedimentation rate) and biochemical blood parameters: albumins, alpha-1, alpha-2, beta- and gamma-globulins, urea, uric acid, creatinine, glucose, sialic acids, alkaline phosphatase, pseudocholinesterase, amylase, alanine and aspartic transaminases, medium-weight molecules, lipids in general, high-, low-, and very-low-density lipoprotein cholesterol, diene conjugates, malondialdehyde, catalase, and erythrocyte superoxide dismutase were determined. The analyzes were carried out according to the instructions described in the manuals (Bazarnova AG & Gette ZP, 1994; Goryachkovskiy AM, 1998). The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets. The reference values are taken from the manuals (Khmelevskyi YV & Usatenko OK, 1987; Perederiy VG et al., 1995; Goryachkovskiy AM, 1998), instructions as well as database of the Truskavetsian Scientific School of Balneology.

Data collection and analysis / Statistical analysis. Statistical processing performed using a software package "Microsoft Excell" and "Statistica 6 StatSoft Inc".

Results

Judging by the average values (M±SE), the observed contingent was characterized by a level of physical working capacity in the lower zone of the narrowed (±1σ) norm (2,28±0,16 W/kg or -0,44±0,18σ), which practically did not change under the influence of balneotherapy (-0,26±0,21 W/kg or -0,29±0,24σ). However, the analysis of individual changes revealed a different picture. In particular, in 10 (45,5%) patients, reduced fitness (1,92±0,23 W/kg or -0,84±0,25σ) was completely normalized (2,57±0,19 W/kg or -0,12±0,21σ; change is +0,65±0,18 W/kg or +0,73±0,20σ). At the same time, in 12 (54,5%) patients, the normal level of fitness (2,57±0,20 W/kg or -0,11±0,22σ) fell to the lower zone of the narrowed norm (1,55±0,13 W/kg or -1,26±0,15σ; change is -1,02±0,13 W/kg or -1,14±0,15σ).

In order to make a correct comparison, the individual actual values (more precisely, their changes) of the Variables (V) were transformed into Z-scores according to the classical formulas: Z = (V/R - 1)/Cv = (V - R)/SD. Two pairs of patterns were created based on significantly different changes (Figs. 1 and 2).

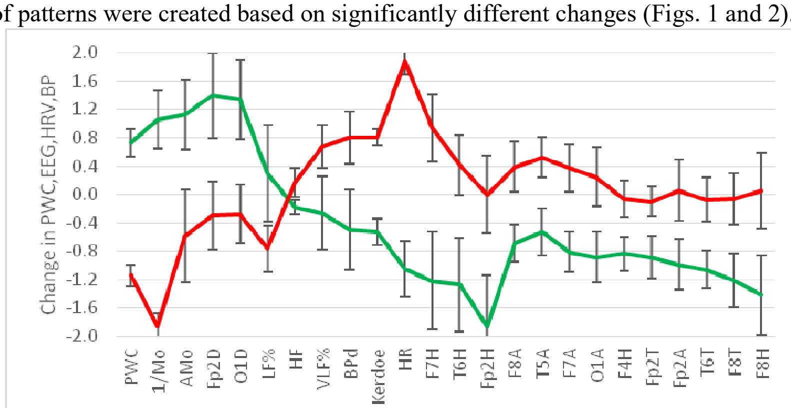


Fig. 1. Patterns of accompanying changes (Z±SE) in EEG, HRV and diastolic blood pressure parameters in cases of PWC decrease and increase

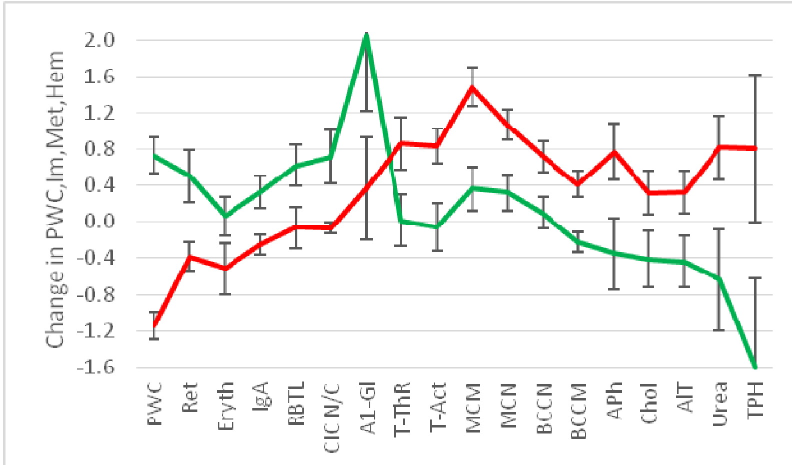


Fig. 2. Patterns of accompanying changes (Z±SE) in immune, erythron, hemostasic and metabolic parameters in cases of PWC decrease and increase

At the next stage of analysis, accompanying changes in parameters are grouped into four clusters (Fig. 3). The first cluster displays unidirectional changes of 11 parameters (see the list in the appendix) with PWC, and the second cluster - reciprocal changes of 15 parameters. In the third cluster, 6 parameters are collected, which do not change when the PWC increases, but increase when it decreases. Conversely, 7 parameters of the fourth cluster decrease when PWC increases, while they remain unchanged when it decreases.

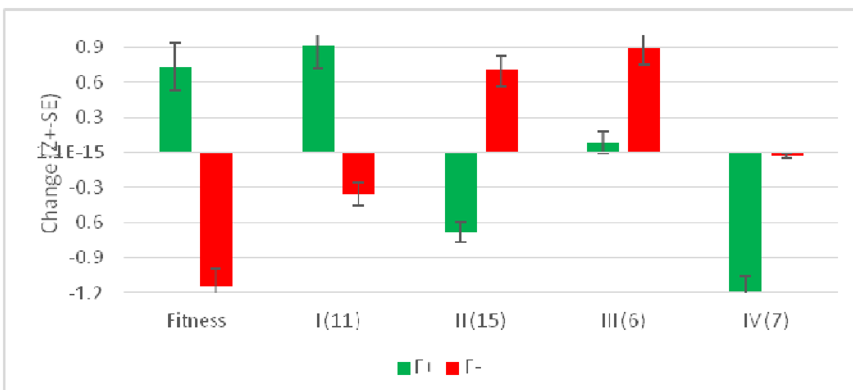


Fig. 3. Clusters of accompanying changes (Z±SE) in parameters (number in parentheses) in cases of PWC increase or decrease

In order to identify parameters whose changes under the influence of balneotherapy are characteristic of two cluster of changes in fitness, their discriminant analysis was carried out (Klecka WR, 1989). The forward stepwise program included in the discriminant model, together with PWC by definition, only 8 parameters: 5 **EEG** as well as one representative each of **HRV**, **metabolism** and **immunity** (Tables 1 and 2). A number of other parameters deserve attention, which also carry discriminative information, but were left out of the model, probably due to its duplication/redundancy.

Table 1. Summary of the analysis of Discriminant Functions in relation to the changes in PWC and accompanying parameters

Step 9, N of vars in model: 9; Grouping: 2 grps; Wilks' Λ: 0,0368; approx. $F_{(9,1)}$=35; p<10^{-6}

Variables currently in the model	Clusters (n) of changes in Fitness		Parameters of Wilks' Statistics					
	F+ (10)	**F- (12)**	Wilks' Λ	Par-tial Λ	F-re-move (1,6)	p-level	Tole-rancy	Refe-rence Cv
Physical Working Capacity, W/kg	1,92 +0,67	2,57 -1,02	0,180	0,204	46,7	10^{-4}	0,2717668	2,67 0,333
VLF PSD, %	50,9 -4,8	38,5 +10,9	0,057	0,648	6,51	0,025	0,425	51,9 0,325
O1-α PSD, %	37,0 -18,5	41,1 +5,2	0,037	0,999	0,01	0,936	0,106	42,0 0,495

F4 Entropy	0,924 -0,099	0,863 -0,009	0,051	0,720	4,66	0,052	0,534	0,851 0,139
Bactericidal Capacity of Monocytes, 10^9 Bacteria/L	0,92 -0,32	0,51 +0,61	0,060	0,617	7,45	0,018	0,475	2,19 0,672
Alanine transaminase, µM/h•L	0,36 -0,09	0,32 +0,06	0,066	0,559	9,47	0,010	0,391	0,39 0,496
O1-δ PSD, %	15,8 +20,6	24,2 -4,2	0,053	0,699	5,18	0,042	0,310	23,5 0,655
F7-α PSD, %	28,2 -11,7	23,1 +5,4	0,056	0,652	6,40	0,026	0,140	27,6 0,522
T5-α PSD, %	30,1 -9,6	27,4 +9,4	0,041	0,892	1,45	0,252	0,110	35,1 0,516
Variables currently not in the model	**F+ (10)**	**F- (12)**	Wilks Λ	Par-tial Λ	F to enter	p-level	Tole-rancy	Refer Cv
Alpha-1 globulins, g/L	5,10 +1,30	5,53 +0,24	0,034	0,922	0,93	0,356	0,799	2,93 0,217
Urea, mM/L	5,83 -0,65	5,42 +0,83	0,036	0,969	0,36	0,562	0,506	5,57 0,281
Mode HRV, msec	891 +106	963 -187	0,034	0,924	0,90	0,363	0,138	875 0,115
HF PSD, $msec^2$	122 -52	86 +70	0,037	0,994	0,07	0,801	0,617	446 0,754
Amplitude of Mode HRV, %	73,5 +13,0	68,6 -6,4	0,035	0,945	0,64	0,442	0,365	39,3 0,300
LF PSD, %	33,9 +3,6	42,8 -8,3	0,036	0,990	0,11	0,749	0,097	27,2 0,381
Fp2 Entropy	0,862 -0,209	0,804 0,000	0,036	0,967	0,38	0,550	0,328	0,835 0,135
Fp2-α PSD, %	31,8 -14,7	29,6 +0,8	0,036	0,986	0,16	0,695	0,374	32,9 0,448
T6 Entropy	0,802 -0,157	0,757 +0,052	0,034	0,936	0,76	0,403	0,434	0,825 0,149
T6-θ PSD, %	8,8 -4,3	8,0 -0,3	0,037	0,992	0,09	0,773	0,342	8,6 0,474

Notes. In each cluster column, the first line is the initial average, the second – average change in variables. The subject of discriminant analysis are changes only.

Table 2. Summary of Stepwise Analysis for Variables. The variables are ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Physical Working Capacity, W/kg	59,0	10^{-6}	0,253	59,00	10^{-6}
VLF PSD, %	8,84	0,008	0,173	45,48	10^{-6}
O1-α PSD, %	5,42	0,032	0,133	39,18	10^{-6}
F4 Entropy	3,42	0,082	0,111	34,20	10^{-6}
Bactericidal Capacity of Monocytes, 10^9 B/L	4,43	0,051	0,087	33,76	10^{-6}
Alanine transaminase, µM/h•L	3,95	0,065	0,069	33,98	10^{-6}
O1-δ PSD, %	2,09	0,170	0,060	31,54	10^{-6}
F7-α PSD, %	5,80	0,032	0,041	37,78	10^{-6}
T5-α PSD, %	1,45	0,252	0,037	34,90	10^{-6}

The identifying information contained in the 9 discriminant variables is condensed into a single root ($r^*=0,981$; Wilks' $\Lambda=0,0368$; $\chi^2_{(9)}=51$; $p<10^{-6}$). Calculating the values of discriminant roots for each patient by the raw coefficients and the constant (Table 3) allows visualization of each person in the information space of root (Fig. 4).

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

	Coefficients		
Variables currently in the model	**Standar-dized**	**Struc-tural**	**Raw**
Physical Working Capacity, W/kg	1,743	**0,336**	3,442
O1-δ PSD, %	1,004	**0,104**	0,041
Bactericidal Capacity of Monocytes, 10^9 B/L	-0,914	**-0,149**	-1,440
F7-α PSD, %	1,608	**-0,115**	0,106
T5-α PSD, %	-1,009	**-0,105**	-0,055
F4 Entropy	-0,738	**-0,094**	-7,529
Alanine transaminase, µM/h•L	-1,082	**-0,091**	-6,507
O1-α PSD, %	-0,075	**-0,088**	-0,003
VLF PSD, %	-0,927	**-0,085**	-0,049
	Constant		0,934

It can be seen (Figs. 4 and 5) that the two clusters of patients differ very clearly from each other not only in terms of changes in PWC, but also in terms of changes in the set of selected parameters.

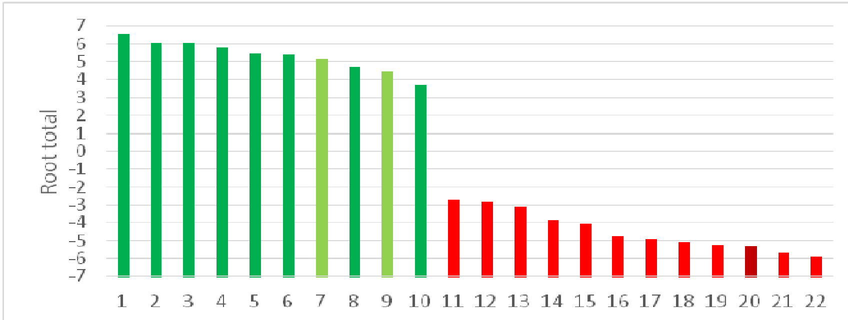


Fig. 4. Scattering of individual values of the discriminant root of changes in Fitness and accompanying EEG, immune and metabolic parameters of patients (Squared Mahalanobis distance=96,0; $F_{(9,1)}$=34,9; $p<10^{-6}$). Three women are highlighted in shades of colors

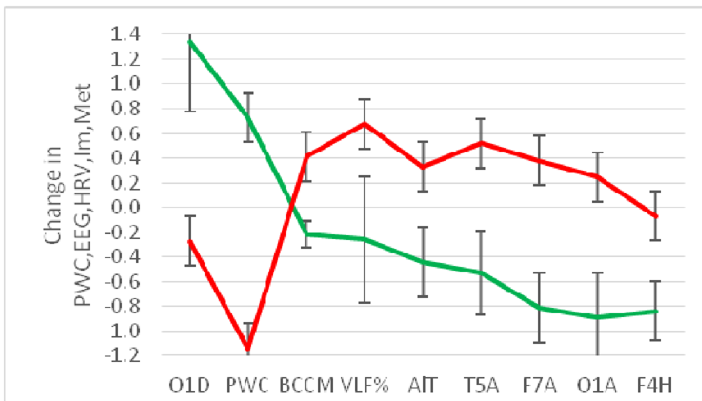


Fig. 5. Patterns of accompanying changes (Z±SE) in characteristic EEG, HRV, immune and metabolic parameters in cases of PWC decrease and increase

In particular, the PSD of the delta rhythm generated by neural structures projecting to the O1 locus changes unidirectionally with the PWC. Instead, the activity of structures projected to the same locus that generate alpha rhythm, as well as the alpha rhythm generating structures projected to loci F7 and T5, as well as PSD entropy in locus F4, change opposite to changes in PWC. Changes opposite to PWC occur on the side of relative PSD of VLF band HRV, alanine transaminase and bactericidal capacity of monocytes of blood.

Selected discriminant variables were used to identify the affiliation of a patient to a particular cluster. This goal of discriminant analysis is realized with the help of classification functions (Table 4). Classification accuracy is 100%.

Table 6. Coefficients and constants of classification functions

Clusters	F+	F-
Variables	p=,455	p=,545
Physical Working Capacity, W/kg	14,52	-19,21
VLF PSD, %	-0,230	0,249
O1-α PSD, %	-0,060	-0,033
F4 Entropy	-37,65	36,11
Bactericidal Capacity of Monocytes, 10^9 B/L	-6,051	8,059
Alanine transaminase, µM/h•L	-30,49	33,26
O1-δ PSD, %	0,202	-0,200
F7-α PSD, %	0,430	-0,606
T5-α PSD, %	-0,148	0,388
Constants	-11,00	-15,60

Since the initial levels of PWC in both groups differed significantly, it would be interesting to analyze the initial and final levels of other discriminant variables (Fig. 6).

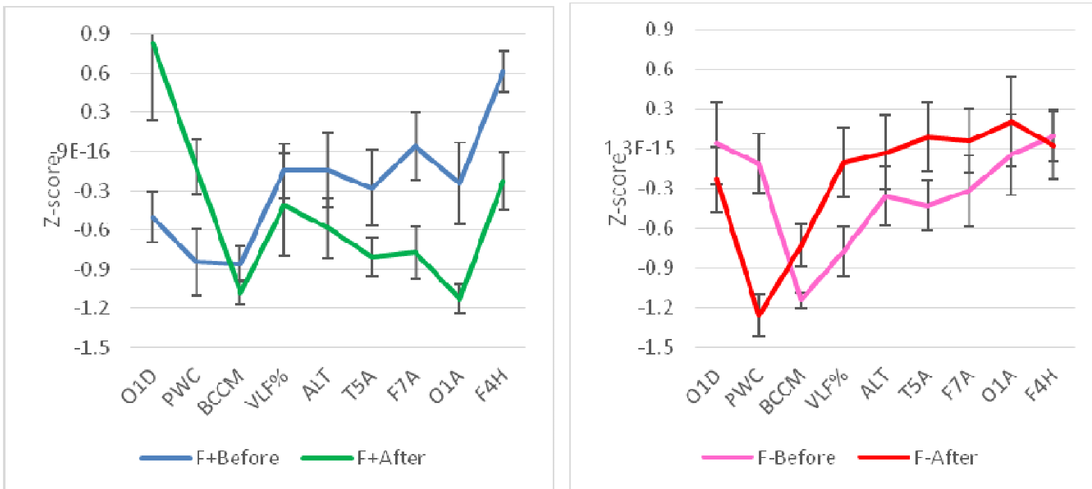


Fig. 6. Patterns of initial and final levels (Z±SE) of PWC and accompanying characteristic EEG, HRV, immune and metabolic parameters in cases of PWC increase (left) or decrease (right)

It turns out that the normalization of reduced PWC is accompanied by an increase in O1-δ PSD from the lower normal zone to the upper one, instead, a decrease in the normal levels of other EEG and HRV parameters and a slight deepening of the suppression of monocyte/macrophage bactericidal activity.

In cases of a decrease in the normal level of PWC, the lower limit levels of HRV&EEG parameters are completely normalized, and the suppressed bactericidal activity increases within the lower normal zone.

Discussion

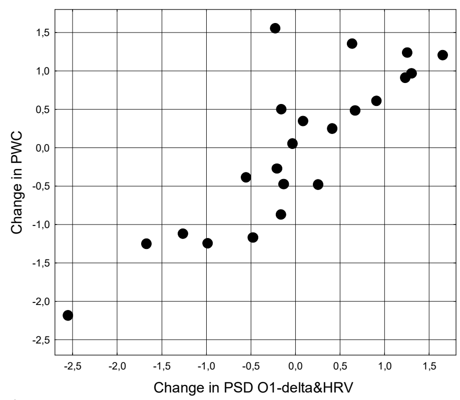
Naftussya bioactive water is the main therapeutic factor of the international spa of Truskavets' (Ukraine). Unlike classic mineral waters, its physiological and therapeutic activity is determined not by salts and gases, but by organic substances and autochthonous microbes (Yessypenko BYe, 1978; Yessypenko BYe, 1981; Yaremenko MS et al., 1989; Ivassivka SV, 1997; Ivassivka SV et al., 1999; Bilas VR & Popovych IL, 2009; Popovych IL et al., 2009; Popovych IL, 2011; Popovych IL et al., 2017; Popovych IL et al., 2018; Zukow W et al., 2020; Popovych IL et al., 2021; Popovych IL et al., 2022).

Approximately 2/3 of the mass of organic substances in Naftussya water is leached from water-bearing petroleum rock, which is reflected in its name (naphta/ναφθα means petroleum in Greek), and 1/3 are the products of their biotransformation by hydrocarbon-oxidizing, sulfate-reducing and thione microbes (Yaremenko MS et al., 1989; Ivassivka SV, 1997). Another source of organic substances, in particular phenols, are fallen leaves on the surface of the deposit (Ivassivka SV et al., 1994). We adduce data by Dats'ko OR et al. (2008) about organic compounds (in mg/L) Naftussya water obtained by Solid Phase Extraction method and mass-spectroscopy by using as Sorbents Tenacle GC 60/80 and Polysorb-2. Paraffins 4,10 and 4,20; monoolefins 1,67 and 1,75; dienes and monocycloolefins 0,84 and 0,85; alkylbenzene 1,55 and 1,54; alkenylbenzene 0,47 and 0,46; esters of aromatic acids 1,32 and 1,33; alkyl phenols 1,14 and 1,14; carboxylic (fatty) acids 1,13 and 1,14; polyaromatic hydrocarbons 0,077 and 0,059; sulfur-containing connections 0,30 and 0,31; alkylnaphthalenes 0,53 and 0,53; unidentified polyaromatic hydrocarbons 0,19 and 0,19; connections required subsquent identification 0,48 and 0,50 correspondingly.

At least some of the listed organic substances (alkylbenzene, alkenylbenzene, alkylnaphthalenes, alkyl phenols, esters of aromatic acids, polyaromatic hydrocarbons) are, obviously, agonists of aryl hydrocarbon receptors (AhR), which are expressed by almost all types of cells of living organisms, starting from unicellular. Although the AhR was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants, the activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the endocrine and immune response (Quintana FJ & Sherr DH, 2013; Esser C & Rannug, 2015; Murray IA & Perdew GH, 2020). Kimura E & Tohyama C (2017) analyzed AhR mRNA expression in the brains of mice. The mRNA was expressed in the hippocampus, cerebral cortex, cerebellum, olfactory bulb. These results reveal temporal and spatial patterns of AhR mRNA expression in the mouse brain, providing the information that may contribute to the elucidation of the physiologic and toxicologic significance of AhR in the developing brain. Although AhR expression decreases from the embryonic period into adult life (Kimura E & Tohyama C, 2017), several physiological functions remain in the adult brain, which include the regulation of neurotransmitter levels, blood-brain barrier functions, and immune responses (Chen WC et al., 2019). Therefore, the ability of Naftussya water organic substances to directly affect CNS neurons via AhR is very real. Another possible mechanism of effect of Naftussya water on the brain is AhR irritation of afferent terminals n. vagus in the intestinal mucosa. By the way, similar AhR agonists are also present in mineral water for baths, so it is likely that they irritate the receptors of the afferent nerves of the skin (more about this in the review: Popovych IL, 2022). It is here that we consider it necessary to note that although the calculated submaximal PWC is considered as an indicator of cardiorespiratory fitness (Finger JD et al., 2013), in reality it reflects the reaction of the autonomic nervous system to muscle load, which, in turn, is strong, but still not absolutely complete, correlates with VO_2max as a real indicator of cardiorespiratory fitness. By the way, the correlation is significantly affected by the use of adreno- and/or cholinergic blockers, as well as autonomic dysfunction, which occurred in the observed contingent as a manifestation of post-radiation encephalopathy. In particular, the marker of sympathetic tone AMo (reference level 39,3±2,2%) at rest was 73,5±8,6% and 68,6±5,9%, and the marker of vagal tone MxDMn (reference level 173±9 msec) - 79±23 msec and 108±11 msec in cluster F+ and F-, respectively, that is, there was a pronounced sympathotonic shift of the sympatho-vagal balance. Interestingly, the Mode as a marker of circulating catecholamines did not differ from the reference level (875±18 msec), being 891±43 msec and 963±37 msec, respectively. Temporal and spectral HRV markers are not considered in this context, since they were not recorded immediately after exercise. The autonomic response to muscle load was paradoxical: AMo decreased to 45,8±7,0% and 35,6±6,0%, while MxDMn increased to 207±35 msec and 219±37 msec in the F+ and F- cluster, respectively. This indicates asympathicotonic autonomic reactivity.

Judging by the schemes of Winkelmann T et al (2017), on loci O1, F7 and T5 are projected lingual gyrus, superior frontal gyrus and transverse temporal cortex of the left hemisphere respectively, and on locus F4 – caudal anterior cingulate cortex of the right hemisphere, the thickness of which is positively correlated (r=0,48; 0,39; 0,51; and 0,55 respectively) with vagally mediated HRV (HF band).

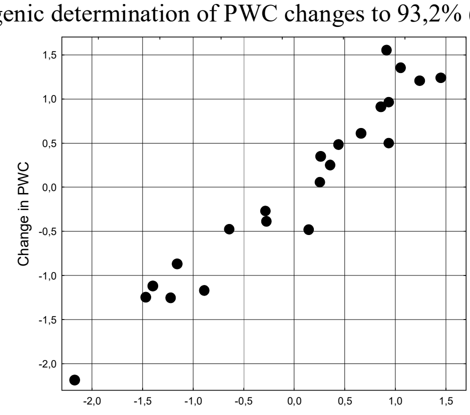
We also found a positive correlation between balneofactor-induced changes in O1-δ PSD at rest and vagal tone after load (r=0,64), but not at rest (r=-0,05). Changes in vagal tone after load, in turn, are positively correlated with changes in PWC (r=0,82). On the other hand, the correlations of O1-δ PSD with sympathetic tone are opposite (r=-0,51), as is the latter with PWC (r=-0,76). Taken together, changes in EEG&HRV parameters determine changes in PWC by 71,3% (Fig. 7).



R=0,845; R^2=0,713; $\chi^2_{(3)}$=23; p<10^{-4}; Λ Prime=0,287

Fig. 7. Scatterplot of canonical correlation between changes in relative PSD O1-δ at rest and HRV after load (X-line) and PWC (Y-line)

In addition, a positive correlation was found between changes in O1-δ PSD and sympathetic tone (r=0,67), as well as circulating catecholamines (r=0,57) at rest. Additional inclusion of these variables in the canonical root increases the degree of neurogenic determination of PWC changes to 93,2% (Fig. 8).



R=0,965; R^2=0,932; $\chi^2_{(5)}$=47; p<10^{-6}; Λ Prime=0,068

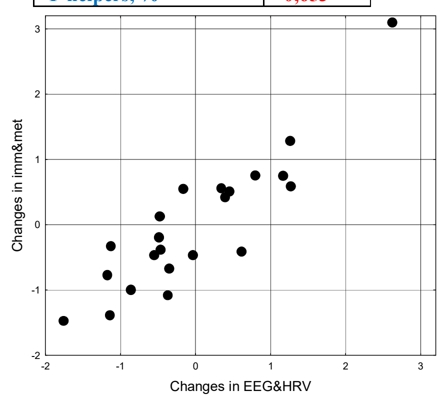
Fig. 8. Scatterplot of canonical correlation between changes in EEG&HRV parameters (X-line) and PWC (Y-line)

Therefore, the opposite effects of balneofactors on PWC that we found are realized through their opposite effects on the basal activity of δ-rhythm-generating neurons of the lingual gyrus of the left hemisphere.

On the other hand, changes in the same cortical structures are positively correlated with changes in the blood level of reticulocytes (r=0,49) and the reaction of transformation of T-lymphocytes into blasts (r=0,33), but negatively with urea (r=-0,41), theophylline-resistant T-lymphocytes (r=-0,46) and bactericidal capacity of blood monocytes (r=-0,44) levels. Because erythrogenesis is closely related to immunogenesis, reticulocytes can be considered an immune parameter. The realization of regulatory effects of the cortex is carried out, apparently, through the mediation of vagal and sympathetic nuclei of the brain stem (Tracey KJ, 2007; Palma JA & Benarroch EE, 2014; Chang EH et al., 2019; Mo J et al., 2019; Popovych IL, 2022). Taken together, neurogenic changes determine changes in immunity parameters and urea by 79,8% (Table 7 and Fig. 9).

Table 7. Factor structure of neural and immune-metabolic canonical roots

Left set	**R**
1/Mo HRV, msec⁻	**-0,967**
Kerdoe's VI, units	**-0,892**
O1-δ PSD, %	**0,720**
AMo HRV, %	**0,608**
Right set	**R**
Reticulocytes, %	**0,791**
RBTL, %	**0,499**
Urea, mM/L	**-0,690**
BCCM, 10^9 B/L	**-0,667**
T-helpers, %	**-0,653**



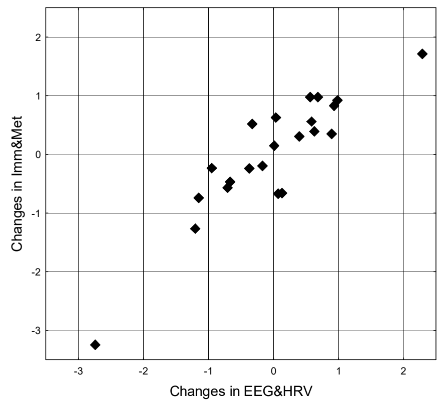
R=0,893; R^2=0,798; $\chi^2_{(20)}$=32; p=0,044; Λ Prime=0,136

Fig. 9. Scatterplot of canonical correlation between changes in neural (X-line) and immune&metabolic (Y-line) parameters

However, contrary to expectations, the relationships between changes in the activity of α-rhythm-generating neurons located in the same area of the cortex, as well as in superior frontal gyrus and transverse temporal cortex of the left hemisphere as well as in entropy in caudal anterior cingulate cortex of the right hemisphere, on the one hand, and HRV parameters, on the other, showed the opposite sign. In our opinion, the only rational explanation for solving the contradictions can be the assumption that the vagotonic effect of the mentioned regions of the cortex is determined not so much by their thickness, but by the activity of the δ-rhythm-generating neurons localized there. Naturally, neuro-immune-metabolic connections turned out to be opposite. It is interesting that the additional inclusion of new EEG parameters in the factor structure of the canonical root (Table 8) almost does not increase the degree of neurogenic determination of changes in immunity and metabolism parameters (Fig. 10).

Table 8. Factor structure of expanded neural and immune-metabolic canonical roots

Left set	**R**
1/Mo HRV, msec⁻	**0,934**
Kerdoe's VI, units	**0,867**
F7-α PSD, %	**0,640**
AMo HRV, %	**0,589**
O1-α PSD, %	**0,232**
O1-δ PSD, %	**-0,690**
Right set	**R**
Reticulocytes, %	**-0,811**
Urea, mM/L	**-0,665**
T-helpers, %	**0,755**
BCCM, 10^9 B/L	**0,613**
RBTL, %	**0,499**



R=0,904; R^2=0,817; $\chi^2_{(24)}$=31; p=0,042; Λ Prime=0,091

Fig. 10. Scatterplot of canonical correlation between changes in all neural (X-line) and immune&metabolic (Y-line) parameters

Somatosensory neurons, innervating the skin, and visceral neurons, innervating the gastrointestinal track, enter the spinal cord via the dorsal horn. In the spinal cord central axonal terminals of these neurons make synaptic contacts with interneurons and relay neurons transmitting the signals to the brainstem and other brain areas. Vagus sensory neurons originate in the nodose ganglia and innervate gastrointestinal tract. Vagal afferent neurons, innervating the gastrointestinal tract are in position to detect gastrointestinal bacteria and signal the brain regions, including vagal and sympathetic nuclei and cortex. Activation of vagal afferents by bacterial products is mediated by TL-4 receptors expressed on these neurons, or via chemosensory cells in the associated vagal paraganglia (review: Chavan SS et al., 2017). The above provides grounds for the assumption that the autochthonous microflora of Naftussya bioactive water acts in a similar way. Another balneofactor - mineral water for a bath - excites skin receptors, information (impulses) from which enters the same nerve centers. In this excellent review is described in detail also the anatomy of efferent autonomic neurons with a role in immune regulation. Therefore, both the chemical factors of mineral bath applied to the skin and the chemical and bacterial (antigenic) factors of Naftussya bioactive water applied to the mucosa of the digestive tract eventually change the activity of the nervous structures of the central autonomic network, which, in turn, modulate the state of the endocrine and immune systems and metabolism. This conclusion fits into the concept of the neuro-endocrine-immune complex (Korneva EA et al., 1993; Korneva EA, 2020; Khaitov RM, 2005; Sternberg EM, 2006; Nance DM & Sanders VM, 2007; Uchakin PN et al., 2007; Popovych IL, 2009; Thayer JF & Sternberg EM, 2010) and the functional-metabolic continuum (Gozhenko AI, 2016).

Back in 1997, Yaremenko MS et al (1997) showed that Naftussya water from the Zbruchanian field activates E-rosette formation in vitro. When repeating the experiment using Naftussya water from the Truskavetsian deposit from different wells and different periods of aerobic storage, Zavyalova OR et al (2001) found both stimulating and suppressive effects on human T-lymphocytes in vitro. Neither the first nor the second group of authors were able to explain the mechanism of the immunotropic effects of Naftussya water in vitro, that is, without the participation of neurotransmitters and hormones, which were already known at the time thanks to Solomon's GF (1987) discovery. Now they are explained by the influence of their organic substances on AhR of immunocytes as well as of autochthonous microflora on toll-like receptors (TLR) of immunocytes. It is interesting that the expression of Ah and TL receptors is interconnected (Murray IA & Perdew GH, 2020).

A direct effect of Naftussya water on immunocytes, in particular T-lymphocytes, is also possible due to fatty acids present in its composition (concentration 40-60 μeqv/L), among which short-chain (7 carbons, 230 daltons) and long-chain (17 carbons, 340 daltons) (Ivassivka SV, 1997). The concentration of fatty acids is comparable both to those used in in vitro immune experiments and those in blood (review: de Jong AJ et al., 2014). Authors present possible mechanism involved in the modulatory effects of fatty acids in T-cells. Free fatty acids enter T-cells through currently unknown mechanisms. Low concentration of free fatty acids are incorporated in phospholipids, while uptake of glucosesustains the formationof triacylglycerol and cholesterol esters. In addition, ree fatty acidsinduce proliferation, lactate and **cytokine** production by T-cells.

Cytokines released by activated immunocytes, primarily IL-1, also excite vagal terminals (review: Chavan SS et al., 2017), in addition to their excitation by AhR agonists.

Thus, modulating effects of drinking medicinal waters (Naftussya, Sofiya, Hertsa et al) on the neuro-endocrine-immune complex of both rats and humans (Popovych IL et al., 2003; Kostyuk PG et al., 2006; Popovych IL, 2011; Kozyavkina OV et al., 2015; Kul'chyns'kyi AB et al, 2016; 2017; Popovych AI, 2018; Popovych AI, 2019; Popadynets' O et al., 2020; Hrytsak MV et al., 2022; Popovych IL, 2022) are explained by the effect of their organic substances on AhR of neurons, endocrinocytes and immunocytes as well as of autochthonous microflora on Toll-like receptors (TLR) of immunocytes of GALT (Ruzhylo SV et al., 2021; Popovych IL et al., 2022).

In conclusion, we will say that one gets the impression that a decrease in fitness under the influence of balneofactors is compensated by their increase in bactericidal activity, while the body "pays" for the increase in fitness by weakening it. This is consistent with the long-known principle of the "physiological price" of adaptation (Meerson FZ, 1991) as well as with the textbook fact of a decrease in athletes' resistance to a banal infection at the peak of cardiorespiratory fitness.

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Accordance to ethics standards

Tests in patients are carried out conducted in accordance with positions of Helsinki Declaration 1975 and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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APPENDIX

Clusters of accompanying changes in EEG, HRV, immunity, hemostasis, erythron and metabolism parameters with opposite changes in fitness

Variables	F+ (10)	F- (12)	Abbre-viation
Physical Working Capacity	**+0,73**	**-1,14**	PWC
Cluster I			
O1-δ PSD	**+1,34**	**-0,28**	O1D
Fp2-δ PSD	**+1,40**	**-0,29**	Fp2D
Amplitude of Mode HRV	**+1,13**	**-0,59**	AMo
LF PSD	**+0,30**	**-0,77**	LF%
BTR T-Lymphocytes on PhHA	**+0,62**	**-0,06**	RBTL
CICnorm/CICcold ratio	**+0,71**	**-0,36**	CIC N/C
Immunoglobulins A	**+0,33**	**-0,24**	IgA
Reticulocytes of Blood	**+0,50**	**-0,38**	Ret
Erythocytes of Blood	**+0,06**	**-0,52**	Erhyth
Alpha-1 globulins as antiplasmin	**+2,04**	**+0,37**	A1-Gl
Tolerance to heparin as antithrombin	**+1,59**	**-0,81**	TPH
Cluster II			
F7 Entropy	**-1,22**	**+0,94**	F7H
T6 Entropy	**-1,27**	**+0,42**	T6H
F7-α PSD	**-0,81**	**+0,38**	F7A
F8-α PSD	**-0,69**	**+0,39**	F8A
T5-α PSD	**-0,53**	**+0,52**	T5A
O1-α PSD	**-0,89**	**+0,25**	O1A
VLF PSD	**-0,26**	**+0,68**	VLF%
1/Mode HRV	**-1,05**	**+1,87**	1/Mo
HF PSD	**-0,18**	**+0,17**	HF
Blood Pressure Diastolic	**-0,50**	**+0,80**	BPd
Heart Rate	**-1,05**	**+1,88**	HR
Kerdoe's Vegetative Index	**-0,53**	**+0,81**	Kerdoe
Alanine transaminase	**-0,44**	**+0,33**	AlT
Alkaline phosphatase	**-0,35**	**+0,77**	APh
Urea	**-0,64**	**+0,61**	Urea
Cholesterol total	**-0,41**	**+0,31**	Chol
Cluster III			
Bactericidal Capacity of Monocytes	**-0,22**	**+0,41**	BCCM
Bactericidal Capacity of Neutrophils	**+0,10**	**+0,72**	BCCN
Microbial Count of Neutrophils	**+0,32**	**+1,07**	MCN
Microbial Count of Monocytes	**+0,36**	**+1,49**	MCM
T-active Lymphocytes	**-0,06**	**+0,84**	T-act
Theophilline resistant T-Lymphocytes	**+0,02**	**+0,83**	T-ThR
Cluster IV			
F4 Entropy	**-0,84**	**-0,07**	F4H
T6-θ PSD	**-1,06**	**-0,07**	T6T
Fp2 Entropy	**-1,86**	**0,00**	Fp2H
Fp2-α PSD	**-0,99**	**+0,06**	Fp2A
Fp2-θ PSD	**-0,89**	**-0,10**	Fp2T
F8 Entropy	**-1,42**	**+0,05**	F8H
F8-θ PSD	**-1,27**	**-0,07**	F8T