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Polivariant change of step-test under the influence of natural adaptogens and their accompaniments

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Abstract

Background. Typically, the step test is used to assess the physical performance that is determined by the condition of the cardio-respiratory system. The latter, in turn, is associated with the nervous, endocrine and immune systems, which collectively determine the level of adaptation of the body. The purpose of this study is to analyze the effect on the step test of natural adaptogens and associated changes in a number of parameters of cardio-respiratory, autonomic nervous and immune systems and metabolism. Material and methods. The target was 80 children of both sexes, ages 10-17, arriving at the clinical sanatorium "Dzherelo" of Truskavets spa from radionuclide contaminated territories. The main subject of the study was the Popovych's step test. State of autonomous nervous and immune systems was assessed as well as

others functional and metabolic tests. **Results**. The contingent surveyed was retrospectively divided into three qualitatively distinct groups. The largest group (53,7%) was the group of children with initially reduced or normal levels of Popovych's Step Test Index (PSI), in which it increased. However, in 28,8% of children PSI remained stable at different initial levels, and in 17,5% even decreased, without falling below the lower limit of normal. Discriminant analysis was subsequently applied to identify constellations of parameters that change are characteristic of each cluster. The program selected as characteristic changes in 2 parameters of autonomous nervous system, 2 functional, 4 metabolic and 3 immune parameters as well as blood trombocytes level. The correct classification of the positive actotropic effect is 93,0%; neutral 65,2%; negative 64,3%, and total correctness is 80,0%. **Conclusion**. Balneotherapy causes multivariate changes in Popopvych's step test, which accompanied by characteristic changes in certain parameters of autonomous nervous and immune systems as well as others functional and metabolic tests.

Key words: Step test, HRV, Immunity, Metabolism, Balneotherapy, Children.

INTRODUCTION

Assessment of the functional state of the cardiovascular system, which limits the level of physical performance, is impossible without the use of load tests. The results of functional tests depend not only on the state of the heart and blood vessels, but also on the state of neuroregulatory mechanisms. Increasing the rhythm frequency during muscular work is mainly due to increased sympathetic effects on the heart and, to some extent, decreased parasympathetic [1,6,7]. The condition of the cardio-respiratory system is associated with the nervous, endocrine and immune systems, which collectively determine the level of adaptation of the body [9,11,16,17]. The purpose of this study is to analyze the effect on the step test of natural adaptogens and associated changes in a number of parameters of cardio-respiratory, autonomic nervous and immune systems and metabolism.

MATERIAL AND METHODS

The target was 80 children of both sexes, ages 10-17, arriving at the clinical sanatorium "Dzherelo" of Truskavets spa from radionuclide contaminated territories (density ¹³⁷Cs 37÷93 kBq/m²). According to γ -spectrometry performed with a human radiation meter "Screensaver-3M", the activity of incorporated ¹³⁷Cs was 1,5÷35 Bq/kg.

The main subject of the study was the step test. As early as 1982, Dushanin SA et al [7] offered a modification of the step test to quantify the level of physical performance. The essence of the test is to rhythmically climb the stairs (height 37 cm) and lower it 90 times within 3 minutes. Both the pulse response to the load (whose power is 2,412 W/kg) and the speed of recovery are taken into account. The authors propose to calculate the index of physical performance (IPP) by the formula:

IPP=(P1+P2+P3-200)/10, where

P1 is pulse (heart rate) for 1 min in position sitting after 5 min of rest;

P2 is heart rate for the first 10 sec after loading, multiplied by 6;

P3 is heart rate for the first 10 sec of the second minute of recovery, multiplied by 6.

However, this formula does not take into account the actual time (t) spent by 90 ascents, so Popovych IL [28] has proposed a variant of the step test index (PSTI):

PSTI=180•100/(HR1+HR2+HR3-200)•t

State of autonomous nervous system was assessed by the method of HRV [2] using the "Cardio" device (Kyiv). However, on the basis of indicators of heart rate sitting and standing calculated Teslenko's index [6]. In the same vein, an Anfimov's corrective test was conducted to assess mental performance, as well as the classic Stange's and Gench's breath holding tests for inspiration and expiration, respectively. The level of hemoglobin was also determined. In the vein of functional-metabolic continuum concept [9] an oral glucose tolerance test (OGTT) was also performed [8].

Lymphocyte phenotypes (markers CD3, CD4, CD8, CD16, CD19) were determined by the method of indirect immunofluorescence reaction of binding of monoclonal antibodies of IHC "Sorbent" with visualization under a fluorescence microscope. Theophylline-resistant and theophylline-sensitive T-lymphocyte subpopulations, serum levels of G, A, M immunoglobulins (radial immunodiffusion method) and circulating immune complexes (polyethylene glycol precipitation method) were also determined.

The state of the phagocytic link of immunity was judged by the activity of phagocytosis (phagocytic index), its intensity (microbial number) and completeness (killing index) against Staphylococcus aureus, with the calculation of bactericidal ability of neutrophils. Serum lysozyme activity was evaluated in the bacteriolysis test of Micrococcus lysodeikticus. To assess local immunity, lysozyme activity in mixed saliva was determined. Used the methodologies in the manuals [14,18,19].

Patients received a standard balneotherapeutic complex, the basis of which was drinking 3 ml/kg Naftussya water three times daily; mineral baths (concentration of Cl-SO₄-Na-Mg salts 20-30 g/L, t 36-37°C, duration 8-10 min) every other day, 8 procedures; ozokerite application to the lesion site (t 45°C, duration 20-30 min) every other day, 8 procedures; dietary nutrition; therapeutic exercise. Adaptogenic properties of the complex and its components have been proven by previous studies of the Truskavetsian Scientific School of Balneology [10-13,16,17,20-27,29].

Results processed by methods of cluster and discriminant analyses, using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Preliminary analysis revealed multidirectional changes of the step-test, just as they are multidirectionally changed under the influence of adaptogens, in particular balneotherapy factors, parameters of various functional systems of the human body and animals [3,4,11,16,17].

For further analysis, the contingent surveyed was retrospectively divided into three qualitatively distinct groups. The largest group (53,7%) was the group of children with initially reduced or normal levels of PSI, in which it increased (Fig. 1), which is quite expected in line with the beneficial effect of balneotherapy on the body [16]. However, in 28,8% of children PSI remained stable at different initial levels, and in 17,5% even decreased, without falling below the lower limit of normal.

In the adult contingent of the Truskavets spa it was shown earlier [28] that due to complex balneotherapy, the index of tachycardia-hypertensive reaction to 1,5 W/kg cycling load in 47,6% of patients increased by 16,6 \pm 2,4%, in 26,2% of persons they did not go beyond \pm 5%, instead of the remaining 26,2% the value of this index decreased by an average of 10,0 \pm 1,0%.

In an experiment on female rats [28], it was found that swimming time to fatigue in 6

animals after 3 weeks of water Naftussya use increased from $13,0\pm1.4$ min to $52,3\pm5,9$ min, in 8 animals from $24,5\pm3,9$ min to $37,3\pm5,9$ min, instead of 4 rats with initial very high performance, adverse changes were observed, ie a reduction in swimming time from 61 ± 7 min to 39 ± 3 min. Thus, in the experimental model, a significant favorable actotropic effect was found in 33,3% of individuals, moderately favorable in 44,4% and unfavorable in 22,2% of cases. At the same time, the causation of actotropic effects by Naftussya water has been proved.



Fig. 1. Individual levels of Popovych's step-test index (PSI) before (X-axis) and after (Y-axis) course of adaptogenic balneotherapy. Green lines indicate normal limits

Discriminant analysis [15] was subsequently applied to identify constellations of parameters that change are characteristic of each cluster. The program (forward stepwise) selected as characteristic changes in 2 parameters of **autonomous nervous system**, 2 **functional**, 4 **metabolic** and 3 **immune** parameters as well as blood **trombocytes** level. Of particular interest are a number of parameters that are not spent in the model (Tables 1 and 2).

Table 1. Discriminant Function Analysis Summary for Accompaniments of Changes in Popovych's Step Test

Popovych's Step Test Step 15, N of vars in model: 15; Grouping: 3 grps Wilks' Lambda: 0,303; approx. $F_{(30,1)}=3,4$; p<10⁻⁶

	Changes	in Popovych's	Step Test	Parameters of Wilks' Statistics			5	
	PST-	PST ₀	PST+	Wilks	Par-	F-	p-	Tole
Changes in Variables	-0,10±0,02	0,00±0,00	+0,13±0,01	Λ	tial	remove	le-	ran-
currently in the model	(14)	(23)	(43)		Λ		vel	cy
Leukocytes total, G/L	-0,12±0,25	-0,29±0,17	+0,36±0,07	,314	,966	1,1	,337	,800
Stange's Test, sec	-2,6±3,6	-2,3±2,5	+5,9±1,8	,350	,867	4,9	,011	,799
Teslenko's Test, points	0,2±0,6	-0,4±0,3	+1,0±0,3	,410	,739	11	,000	,600
Trombocytes, G/L	-4±5	$+11\pm6$	+20±5	,320	,946	1,8	,173	,856
Glucosa a 1h OGTT,	+0,15±0,31	$+0,38\pm0,16$	+0,63±0,10	,314	,964	1,2	,315	,702
mM/L								
Glucosa basal, mM/L	0,00±0,08	$+0,08\pm0,09$	+0,20±0,07	,321	,943	1,9	,158	,192
CIC, units	+18±11	$+14\pm6$	-6±6	,356	,851	5,5	,006	,481
AMo HRV, %	$+2,5\pm2,5$	0,0±2,1	-1,6±1,3	,327	,926	2,5	,090	,696
Pan Lymphocytes, %	$+4,5\pm2,2$	$-1,3\pm1,4$	-0,9±1,3	,325	,933	2,3	,113	,678
Glycemia aft 2h OGTT,	+7,4±4,4	-1,6±4,7	-1,7±3,5	,314	,966	1,1	,333	,077
Phagocytose Index %	+18+3	+8+2	+10+1	358	847	57	005	782
Clucosa a 2h OCTT	$+10\pm 5$ +0.35+0.14	-0.05 ± 0.17	$+10\pm1$ +0.15+0.12	378	,077	2.6	,003	,782
mM/L	+0,55±0,14	-0,03±0,17	+0,13±0,12	,520	,925	2,0	,001	,095
IgA Serum, g/L	+0,15±0,28	-0,15±0,18	$+0,24\pm0,11$,316	,958	1,4	,262	,772
Theoph Sensit T Lymph.,	$-3,6\pm1,2$	+1,6±1,2	$+1,0\pm1,1$,336	,903	3,4	,041	,514
%								
MxDMn HRV, sec	0,00±0,05	$+0,04\pm0,02$	$-0,01\pm0,02$,354	,856	5,3	,007	,619
	PST-	PST ₀	PST+	Wilks	Par-	F to	p-	Tole
Changes in Variables	-0,10±0,02	0,00±0,00	+0,13±0,01	Λ	tial	en-ter	le-	ran-
currently not in the model	(14)	(23)	(43)	207	Λ	(2)	vel	cy
CD8 ⁺ T Lymphocytes, %	-1,8±0,8	$+1,7\pm0,9$	$+1,2\pm0,8$,297	,980	,63	,536	,051
CD16 ⁺ NK Lymphocytes, %	-2,2±2,2	$+2,6\pm1,0$	$+2,3\pm1,0$,302	,997	,09	,915	,319
Theophyl Res T Lymph.,	+7,5±2,9	+3,3±1,8	+3,4±1,8	,297	,979	,65	,525	,668
%								
CD4 ⁺ T Lymphocytes, %	$+4,3\pm1,5$	$+1,3\pm0,9$	$+1,4\pm0,9$,297	,979	,65	,525	,561
Killing Index Neutroph,	+14±2	$+5\pm2$	+7±2	,296	,976	,78	,464	,156
Bactericidity Neutr, 10 ⁹	+7,43±1,47	+4,31±0,77	$+4,87\pm0,70$,301	,993	,21	,807	,328
B/L					-		-	
Hemoglobin, g/L	$+0,45\pm0,57$	-0,11±0,34	$+0,84\pm0,26$,302	,995	,14	,867	,728
Erythroc Sedim Rate,	0,0±0,5	-1,6±0,4	-0,2±0,3	,299	,985	,46	,632	,850
mm/h								
Anfimov's Test, error/2	-1,2±0,9	-2,3±0,5	-0,9±0,3	,297	,981	,62	,543	,732
min								
Hench's Test, sec	+1,6±4,4	-0,1±2,8	$+5,3\pm1,3$,295	,974	,83	,441	,596
Segmented Neutrophils,	-0,9±2,5	+3,4±1,5	-0,9±1,3	,298	,983	,55	,582	,824
CD19 ⁺ B Lymphoeytes %	+0 4+0 8	+1.6+0.6	+0 6+0 4	299	985	47	625	663
CDI D Lymphocytes, 70	, ,, <u>,,</u> ,,,,,,,,,,,,,,,,,,,,,,,,,,,,	· 1,0-0,0	1,0,0±0,7	,2,7,7	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	, T/	,025	,005

 Table 2. Summary of Stepwise Analysis for Accompaniments of Changes in Popovych's

 Step Test. The variables are ranked by criterion Lambda

Changes in Variables currently in	F to	p-	Λ	F-	p-
the model	enter	level		value	level
Leukocytes total, G/L	6,1	,004	,864	6,1	,004
Phagocytose Index, %	4,9	,010	,765	5,4	10-3
Stange's Test, sec	3,5	,037	,701	4,9	10-3
Teslenko's Test, points	4,9	,010	,619	5,0	10-4
Glucosa basal, mM/L	3,1	,050	,570	4,7	10-5
CIC, units	2,6	,084	,532	4,5	10-5
Theophilline Sensitive T Lymph., %	3,0	,057	,491	4,3	10-5
MxDMn HRV, sec	3,3	,042	,448	4,3	10-6
Glucosa after 2h OGTT, mM/L	2,9	,064	,414	4,3	10-6
AMo HRV, %	2,2	,122	,389	4,1	10-6
Pan Lymphocytes, %	3,3	,044	,354	4,1	10-6
Trombocytes, G/L	1,5	,240	,339	3,9	10-6
IgA Serum, g/L	1,6	,218	,324	3,8	10-6
Glucosa after 1h OGTT, mM/L	1,0	,365	,314	3,6	10-6
Glycemia after 2h OGTT, %	1,1	,333	,303	3,4	10-6

Next, the 15-dimensional space of discriminant variables transforms into 2-dimensional space of canonical roots. The canonical correlation coefficient is for Root 1 0,738 (Wilks' Λ =0,303; $\chi^{2}_{(30)}$ =84; p<10⁻⁶) and for Root 2 0,578 (Wilks' Λ =0,666; $\chi^{2}_{(14)}$ =28; p=0,012). The major root contains 70,5% of discriminative opportunities and the minor is 29,5%.

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).

Coefficients	Stan	Standardized		aw
Changes in Variables currently in	Root Root		Root	Root
the model	1	2	1	2
Leukocytes total, G/L	,206	,240	,335	,391
Phagocytose Index, %	-,335	,633	-,035	,066
Stange's Test, sec	,551	,070	,045	,006
Teslenko's Test, points	,881	,186	,448	,095
Glucosa basal, mM/L	,518	-,669	1,215	-1,569
CIC, units	-,717	-,295	-,020	-,008
Theophilline Sensitive T Lymph., %	-,459	-,468	-,074	-,075
MxDMn HRV, sec	-,497	-,541	-3,572	-3,888
Glucosa after 2h OGTT, mM/L	,103	1,551	,133	2,001
AMo HRV, %	-,429	,128	-,047	,014
Pan Lymphocytes, %	-,344	,320	-,043	,040
Trombocytes, G/L	,285	-,239	,009	-,008
IgA Serum, g/L	,306	-,095	,372	-,115
Glucosa after 1h OGTT, mM/L	-,092	-,374	-,116	-,473
Glycemia after 2h OGTT, %	,001	-1,156	,000	-,053
	Constants		-,136	-,453
	E	igenvalues	1,198	,501
	Cum.	Properties	,705	1,000

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

Table 4 shows the correlation coefficients of PST changes (discriminant variables) with canonical discriminant roots and the cluster centroids of both roots.

Table 4. Correlations	Variables-Canonical	Roots and	Roots	Means of	f changes in	Variables
for Clusters						

Changes in Variables currently in	Corre	lations
the model	Variables-Roots	
Root 1 (70,5%)	R 1	R 2
Leukocytes total, G/L	,357	,105
Stange's Test, sec	,316	,002
Teslenko's Test, points	,277	,146
Trombocytes, G/L	,218	-,221
Glucosa after1h OGTT, mM/L	,199	-,134
Glucosa basal, mM/L	,163	-,079
CIC, units	-,262	,042
AMo HRV, %	-,134	,129
Pan Lymphocytes, %	-,108	,339
Glycemia after 2h OGTT, %	-,077	,193
Root 2 (29,5%)	R 1	R 2
Phagocytose Index, %	-,110	,480
Glucosa after 2h OGTT, mM/L	,020	,245
IgA Serum, g/L	,154	,175
Theophiline Sensitive T Lymph., %	,093	-,336
MxDMn HRV, sec	-,109	-,142

The above allows us to comment on Fig. 2 as follows. The localization of points representing children with PST growth in the right area of the axis of the first root reflects the

fact that it is accompanied by an increase in variables that correlate **positively** with the radical (Table 4), namely (Table 1): respiration delay time, improvement orthostatic test, increased blood levels of common leukocytes and platelets, basal glycemia, and one hour after oral glucose loading. On the other hand, the increase in PST is accompanied by a decrease in sympathetic tone and blood content of circulating immune complexes and a tendency to decrease the late hyperglycemic response to the loading of glucose and the proportion of pan-lymphocytes associated with the root **inverse**. We consider the described changes physiologically favorable.



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

On the opposite side are localized projections on the axis of points, which represent children with **stable** PST and its **negative** dynamics, which are mixed, reflecting approximately the same **decrease**/increase in the above variables.

These two groups of children are separated along the axis of the second radical. The upper position of points of children with **negative** PST changes reflects more pronounced, than in the **neutral** group, an **increase** in phagocytosis activity and a positive, as opposed to **negative**, dynamics of IgA levels and a late hyperglycemic reaction to glucose loading, on the one hand, and a **decrease** in the proportion of theophylline sensitive T-Lymphocytes and no increase in vagus tone, while in the other group both variables **increase** - on the other hand.

Taking into account variables that are not included in the model (Table 1), we see that the negative dynamics of PST is accompanied by a decrease in the proportion of blood T-killers and natural killers in combination with an increase in T-helpers and bactericidal neutrophils, ie physiological assessment is ambiguous.

Despite the individual ectopies, on the plane of the two discriminative roots, the members of the three groups are delimited statistically, which is documented by the calculation of Mahalanobis distances (Table 5).

Clusters	PST ₀	PST-	PST+
PST ₀	0,00	4,61	5,40
PST-	2,06	0,00	6,72
	0,025		
PST+	4,26	3,64	0,00
	<10 ⁻⁴	<10 ⁻³	

1 able 5. Squared Manalanobis Distances between Clusters, r -values and pleve	Table 5. S	quared Mahalanok	is Distances	between Clusters	F-values and	p levels
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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).

Table 6. Coefficients and Constants for Classification Functions of Clusters

Changes in Variables currently in the	PST ₀	PST-	PST+
model	p=,288	p=,175	p=,537
Leukocytes total, G/L	-,737	,061	,298
Phagocytose Index, %	,116	,257	,096
Stange's Test, sec	-,047	-,038	,053
Teslenko's Test, points	-,547	-,381	,484
Glucosa basal, mM/L	,252	-3,141	1,535
CIC, units	,034	,018	-,015
Theophilline Sensitive T Lymph., %	,158	,005	-,062
MxDMn HRV, sec	7,241	-,682	-3,569
Glucosa after 2h OGTT, mM/L	-1,479	2,724	,460
AMo HRV, %	,053	,086	-,036
Pan Lymphocytes, %	,038	,126	-,020
Trombocytes, G/L	,012	-,005	,025
IgA Serum, g/L	-,343	-,613	,352
Glucosa after 1h OGTT, mM/L	1,487	,499	,849
Glycemia after 2h OGTT, %	,034	-,076	-,009
Constants	-2,813	-4,842	-2,378

The correct classification of the positive actotropic effect is 93,0%; neutral 65,2%; negative 64,3%, and total correctness is 80,0% (Table 7).

Table 7. Classification Matrix for Clusters

Rows: Observed classifications; Columns: Predicted classifications

	Percent	PST+	PST ₀	PST-
Clusters	Correct	p=,538	p=,287	p=,175
PST+	93,0	40	2	1
PST ₀	65,2	5	15	3
PST-	64,3	2	3	9
Total	80,0	47	20	13

To compare accuracy, we recall that in adult patients were selected for inclusion in model 10 variables from 72 counts (in order of decreasing F-value): tachycardia-hypertensive load index, Klimov atherogenic factor, cholesterol content VLD LP, sodium content erythrocytes, activity of Na, K-ATPase erythrocyte shadows, level of diastolic and systolic blood pressure; the plasma potassium and urates content, as well as the Ruzhylo-Popovych's contractile activity index of

myocardium. The correct classification of the positive actotropic effect is 81,0%; neutral 60,0%; negative 72,7%, and total correctness is 75,6% [28].

In the next article, the possibility of predicting the diversity of actotropic effects of adaptogenic balneotherapy will be considered.

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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 parent of 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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