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FORECASTING OF MULTIVARIANT CHANGES IN STEP TEST UNDER THE INFLUENCE OF NATURAL ADAPTOGENS

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Summary

Background. Earlier, we found the multivariant nature of the Popovych's step test change in children after a course of adaptogenic balneotherapy. In particular, the step test improved in 53,7% of children, remained unchanged in 28,8%, and worsened in 17,5%. The purpose of this study is to identify predictors that allow us to forecast each variation of the step test. **Material and methods**. Object of observation was 80 children of both sexes, ages 10-17, arriving at the clinical sanatorium "Dzherelo" of Truskavets' spa from radionuclide contaminated territories. The main parameter of the study was the Popovych's step test. State of autonomous nervous, endocrine and immune systems was assessed as well as others functional and metabolic tests. **Results**. Discriminant analysis was subsequently applied to identify constellations of parameters-predictors of each variant of change in step test. The program selected 16 initial parameters as predictors (primarily level of step test, next sex, body mass and temperature, levels of Gench's and Teslenko's tests, vagal tone, erythrocytes sedimentation rate, trombocytes level as well as 7 immune parameters). The correctly of forecasting of the positive actotropic effect is 90,7%; of neutral is 78,3%; of negative is 92,9%, and total correctness is 87,5%. **Conclusion**. Balneotherapy causes multivariate changes in Popopvych's step test, which determined by constellation of 16 initial parameters.

Key words: Step test; HRV; Hormones; Immunity; Metabolism; Balneotherapy; Children; Forecasting.

INTRODUCTION

Earlier, we found the multivariant nature of the Popovych's step test change in children after a course of adaptogenic balneotherapy. In particular, the step test improved in 53,7% of children, remained unchanged in 28,8%, and worsened in 17,5% [34]. The purpose of this study is to identify predictors that allow us to forecast each variation of the step test.

MATERIAL AND METHODS

The object of observation was 80 children (48 boys and 32 girls), ages 10-17, arriving at the clinical sanatorium "Dzherelo" of Truskavets' spa from radionuclide contaminated territories (density ^{137}Cs $37 \div 93$ kBq/m^2). According to γ-spectrometry performed with a human radiation meter "Screensaver-3M", the activity of incorporated ^{137}Cs was $1,5 \div 35$ Bq/kg. No somatic pathology was detected in most children, 14 were diagnosed with chronic cholecystitis/cholangitis or gastroduodenitis, and another 14 were diagnosed with combined pathology.

The main subject of the study was the Popovych's step test [30,32,34]. State of autonomous nervous system was assessed by the method of HRV [1] using the "Cardio" device (Kyiv). Also, on the basis of levels of heart rate sitting and standing calculated Teslenko's index [5]. Among hormones determined Cortisol, Aldosterone, Testosterone and Triiodothyronine (by the ELISA with the use of analyzers "Tecan" and "RT-2100C" and corresponding sets of reagents from "Алкор Био" and XEMA Co., Ltd).

The level of hemoglobin, erythrocytes, erythrocytes sedimentation rate, trombocytes and leukocytes was determined. We counted up Leukocytogram (LCG) (Eosinophils, Stub and Segmentonucleary Neutrophils, Lymphocytes and Monocytes) and calculated its Adaptation Index as well as Entropy by Popovych IL [11,22,25,].

Lymphocyte phenotypes (markers CD3, CD4, CD8, CD16, CD19) were determined by the method of indirect immunofluorescence reaction of binding of monoclonal antibodies of "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 count) 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 [9,19,21].

Anfimov's corrective test was conducted to assess mental performance, as well as the classic Stange's and Gench's breath delaying tests for inspiration and expiration, respectively [25,30,32].

In the vein of functional-metabolic continuum concept [6] an oral glucose tolerance test (OGTT) was also performed.

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

RESULTS

In order to identify predictors of the three variants of the step test change, all registered initial parameters of children were subjected to discriminant analysis by the forward stepwise method [10].

The program included only 16 initial parameters in the discriminant model (tables 1 and 2). These were, in addition to the expected step test index, Teslenko's and Gench's tests, HRV-marker of vagal tone, sex-index as the ratio between boys (0) and girls (1), body weight, normalized by sex and age, platelet count, erythrocytes sedimentation rate as well as seven immune parameters (leukocytes level, relative content of eosinophils, total lymphocytes, populations of natural killers and theophylline-resistant T lymphocytes, intensity of phagocytosis of Staphylococcus aureus by neutrophils and serum IgA level) as well as not expected evening, but not morning body temperature.

Other registered initial parameters of the organism were outside the model. However, 19 of them deserve attention in terms of predicting the nature of the impact of balneotherapy on the step test.

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

Step 16, N of vars in model: 16; Grouping: 3 grps

Wilks' Lambda: 0,2419; approx. $F_{(32,1)}$=4,0; $p<10^{-6}$

	Changes in Popovych's Step Test			Parameters of Wilks' Statistics					
Variables-predictors currently in the model	**PST- (14)**	**PST_0 (23)**	**PST+ (43)**	Wilks Λ	Partial Λ	F-remove	p-level	Tolerancy	**Reference level (30)**
Popovych Step Test, p-ts	**0,71±0,04**	0,63±0,03	0,55±0,01	,289	,838	6,0	,004	,530	0,68±0,03
$CD16^+$ NK Lymphoc, %	**19,7±2,2**	14,2±0,9	14,4±0,7	,287	,843	5,8	,005	,190	15,6±1,5
Teslenko's Test, points	**4,8±0,5**	4,3±0,4	3,2±0,3	,256	,945	1,8	,174	,758	7,0±0,2
Trombocytes, G/L	**280±9**	255±8	250±6	,265	,913	3,0	,060	,704	275±3
Sex Index, points	**0,57±0,14**	0,43±0,11	0,33±0,07	,345	,702	13,2	10^{-4}	,411	0÷1
Body Mass, % of norm	**108,9±4,6**	100,2±2,2	102,2±2,1	,291	,830	6,3	,003	,638	100±1,8
MxDMn as Vagotone, sec	**0,34±0,03**	0,28±0,03	0,29±0,02	,259	,935	2,1	,126	,712	0,22±0,02
Microbial Count, Bac/Ph	**3,8±0,2**	4,0±0,2	4,7±0,2	,255	,949	1,7	,196	,704	7,0±0,3
Pan Lymphocytes, %	**26,6±2,2**	34,9±1,7	33,2±1,2	,253	,957	1,4	,259	,213	35,5±1,1
Theophyl Res T Lym., %	**31,5±1,0**	36,5±2,1	34,8±1,2	,274	,882	4,2	,020	,318	42,0±1,8
Leukocytes total, G/L	5,78±0,35	**6,12±0,26**	**5,31±0,12**	,256	,943	1,9	,163	,865	5,00±0,18
Eryth Sedim Rate, mm/h	6,1±0,7	**7,4±0,7**	**6,2±0,4**	,262	,922	2,6	,082	,585	8,0±0,5
Gench's Test, sec	30,9±2,7	**32,8±3,3**	**26,5±1,6**	,265	,912	3,0	,057	,790	26±1
IgA Serum, g/L	1,45±0,22	**1,57±0,19**	**1,21±0,08**	,254	,951	1,6	,210	,813	1,90±0,18
Body Temperat Even, ^{0}C	36,48±0,06	**36,41±0,05**	**36,52±0,03**	,274	,884	4,1	,022	,607	36,60±0,02
Eosinophiles, %	3,3±0,5	**3,0±0,5**	**3,2±0,4**	,258	,936	2,1	,128	,714	2,75±0,16
Variables-predictors currently not in the model	**PST- (14)**	**PST_0 (23)**	**PST+ (43)**	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy	**Reference level (30)**
Cortisol, nM/L	**726±58**	556±36	604±27	,238	,983	,52	,599	,586	396±19
Theoph Sens T Lym., %	**22,6±0,9**	18,5±1,2	18,6±1,0	,229	,948	1,67	,196	,196	19,3±1,1
$CD8^+$ T Lymphocytes, %	**25,9±0,7**	23,4±0,9	23,2±0,8	,229	,947	1,71	,190	,218	24,7±0,8
CIC, units	**35±7**	50±6	55±5	,239	,987	,41	,664	,353	44±4
Phagocytose Index, %	**50,3±1,8**	55,5±1,4	56,7±1,5	,240	,994	,18	,835	,756	73,5±2,1
Killing Index, %	**46,9±2,1**	53,4±2,1	53,0±2,1	,240	,994	,18	,835	,715	68,6±2,9
Baevski Stress Index, ln	**4,06±0,24**	4,41±0,20	4,46±0,13	,239	,989	,34	,711	,101	4,84±0,08
AMo as Symp. Tone, %	**32,7±3,6**	36,3±2,9	38,6±1,8	,240	,993	,23	,797	,331	39,8±1,6
$CD4^+$ T Lymphocytes, %	**28,0±0,5**	31,1±1,1	30,3±0,7	,229	,947	1,71	,189	,013	33,6±0,9
Pan Lymphocytes, 10^9/L	1,56±0,17	**1,96±0,14**	**1,69±0,09**	,240	,991	,28	,758	,235	2,12±0,25
Entropy of LCG, $•10^3$	606±19	**646±12**	**631±8**	,241	,997	,08	,926	,336	685±11
Morbidity Index	0,50±0,23	**0,74±0,18**	**0,42±0,11**	,236	,977	,71	,494	,564	0
Rod Neutrophils, %	2,9±0,4	**3,9±0,4**	**3,45±0,2**	,241	,998	,05	,954	,584	3,25±0,11
Monocytes, %	3,0±0,4	**4,0±0,4**	**3,45±0,2**	,241	,998	,08	,928	,576	6,0±0,2
Hemoglobin, % of norm	93,1±1,3	**95,1±0,9**	**92,4±0,7**	,239	,987	,41	,667	,535	100±1,8
Popovych's Adaptat Ind	0,72±0,10	**0,69±0,08**	**0,83±0,06**	,239	,990	,31	,736	,788	1,70±0,04
Segmented Neutroph, %	58,4±2,0	**51,0±1,8**	**54,4±1,2**	,237	,978	,69	,506	,520	52,5±1,6
Body Mass, kg	53,5±3,8	**46,1±2,5**	**48,2±1,8**	,222	,980	,61	,548	,204	
Age, years	13,6±0,7	**12,9±0,9**	**13,3±0,3**	,240	,994	,18	,835	,715	

Table 2. Summary of Stepwise Analysis for Predictors of Changes in Popovych's Step Test. The variables are ranked by criterion Lambda

	F to enter	p-level	Λ	F-value	p-level
Popovych Step Test, points	8,9	,0003	,812	8,9	,000325
Sex Index, points	11,0	,0001	,629	9,9	,000000
CD16$^+$ NK Lymphocytes, %	5,8	,0046	,545	8,9	,000000
Theophylline Resistent T Lymph., %	4,3	,0169	,488	8,0	,000000
Leukocytes total, G/L	3,7	,0302	,444	7,3	,000000
Body Mass, % of norm	2,3	,1033	,417	6,6	,000000
Body Temperature Evening, ^{0}C	2,3	,1092	,391	6,1	,000000
Hench's Test, sec	3,5	,0365	,356	5,9	,000000
IgA Serum, g/L	2,1	,1253	,335	5,6	,000000
Trombocytes, G/L	1,5	,2289	,321	5,2	,000000
Erythrocytes Sedimentation Rate, mm/h	1,6	,2145	,307	4,9	,000000
Teslenko's Test, points	1,8	,1810	,291	4,7	,000000
MxDMn HRVas Vagal tone, sec	1,5	,2205	,278	4,5	,000000
Eosinophiles, %	1,7	,1821	,264	4,3	,000000
Microbial Count, Bac/Phag	1,4	,2635	,253	4,2	,000000
Pan Lymphocytes, %	1,4	,2593	,242	4,0	,000000

Next, the 16-dimensional space of discriminant variables transforms into 2-dimensional space of canonical roots. The canonical correlation coefficient is for Root 1 0,790 (Wilks' Λ=0,242; $\chi^2_{(32)}$=99; $p<10^{-6}$) and for Root 2 0,598 (Wilks' Λ=0,642; $\chi^2_{(15)}$=31; p=0,010). The major root contains 75% of discriminative opportunities and the minor is 25%.

Table 3 presents coefficients for discriminant variables. The calculation of the discriminant root values for each child 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 child in the information space of the roots (Fig. 1).

Table 3. Standardized, Structural and Raw Coefficients and Constants for Predictors

Coefficients	**Standardized**		**Structural**		**Raw**	
Variables	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2
Popovych's Step Test, points	,690	,166	**,368**	,115	5,479	1,319
CD16$^+$ NK Lymphocytes, %	,995	-,763	**,265**	-,219	,182	-,140
Teslenko's Test, points	,332	,098	**,263**	,192	,177	,052
Trombocytes, G/L	,383	-,299	**,222**	-,073	,010	-,008
Sex Index, points	1,069	-,183	**,147**	,036	2,179	-,374
Body Mass, % of norm	,626	-,243	**,132**	-,191	,046	-,018
MxDMn HRVas Vagotone, sec	,057	-,498	**,096**	-,146	,456	-4,018
Microbial Count, Bac/Phagocyt	-,015	-,451	**-,225**	-,222	-,013	-,401
Pan Lymphocytes, %	,427	-,491	**-,221**	,298	,053	-,061
Theophyl Resist. T Lymph., %	-,523	,750	**-,104**	,210	-,063	,091
Leukocytes total, G/L	,283	,209	,153	**,378**	,276	,204
Erythroc Sediment Rate, mm/h	-,364	,374	,005	**,255**	-,134	,138
Gench's Test, sec	,301	,392	,119	**,246**	,025	,032
IgA Serum, g/L	,024	,410	,114	**,245**	,034	,585
Body Temperature Evening, ^{0}C	-,318	-,599	-,057	**-,269**	-1,729	-3,263
Eosinophiles, %	,219	-,410	,013	**-,042**	,096	-,181
Eigenvalues	1,656	,557	**Constants**		46,85	122,7
	Discriminant Proportio				75%	25%

The localization in the extreme right zone of the major root (centroid: +2,53) of the cluster of children in whom the step test index decreased under the influence of balneotherapy, reflects, first, their maximally increased levels of natural killers, body weight normalized by sex and age, and vagal tone; secondly, maximally for sampling normal levels of step test and platelets and minimally reduced level of Teslenko's index; thirdly, maximally for sampling ratio of girls/boys; fourthly, maximally reduced levels of phagocytosis intensity, relative content of lymphocytes in general and theophylline-resistant subpopulation in particular.

Also noteworthy are parameters that were not formally included in the model: maximally elevated levels of cortisol as well as subpopulations of theophylline-sensitive and $CD8^+$ T-lymphocytes - on the one hand, and maximally reduced levels of Baevsky's stress index, sympathetic tone, CIC, $CD4^+$ T-lymphocytes as well as activity and completeness of phagocytosis - on the other hand.

The members of the other two clusters list the parameters are smaller/larger (according to the sign of the structural coefficient), and their points are mixed, which reflects the absence of significant differences between them (centroids: +0,20 and -0,93 respectively).

Instead, these clusters are delimited along the axis of the minor root: centroids +1,15 and -0,41 for the "neutral" and "positive" clusters, respectively. The lower position of children in whom the step test index increased reflects their reduced erythrocyte sedimentation rate and IgA level as well as normal levels of leukocytes and Gench's test, while in children with a stable step test index, these parameters are normal or elevated, respectively. On the other hand, in children of a "positive" cluster, the evening body temperature is quite normal, and the level of eosinophils is upper limit, while in a "neutral" cluster, the temperature and eosinophilia are slightly lower.

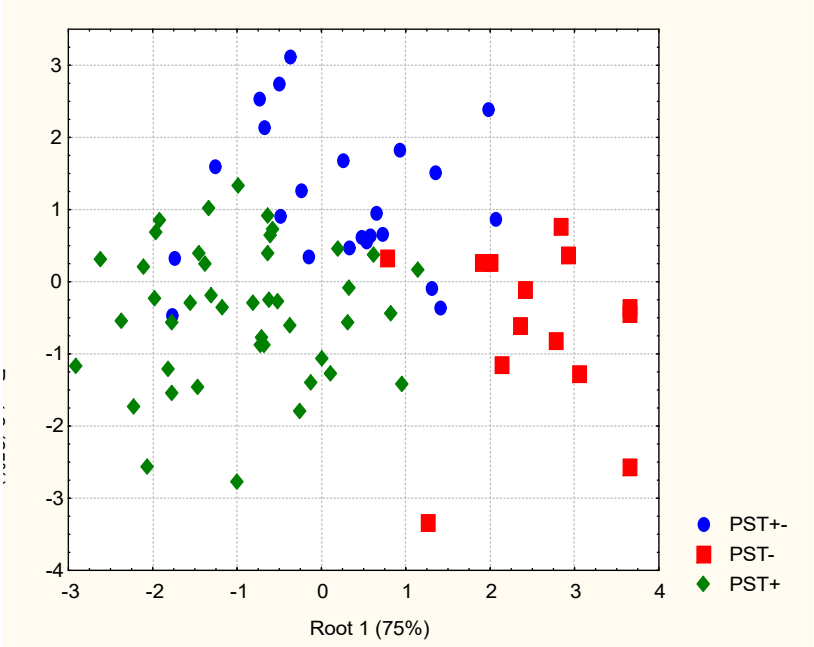

Fig. 1. Scatterplot of individual values of the first and second roots in which condensed information about of the predictors of changes in Popovych's step test of the members of the three clusters

Among the extramodel parameters, the "positive" cluster members have a reduced hemoglobin level, reduced lymphocyte and monocyte proportions in the leukocytogram and reduced entropy of the latter, while the normal proportion of rod neutrophils, while the "neutral" cluster members have a highest level of these parameters. On the other hand, members of the "positive" cluster have a higher level of segment nuclear neutrophils,

Popovych's leukocytary adaptation index, body weight and age, while a lower morbidity index, calculated as the average number of diagnoses in a child (0; 1; 2).

In a set of 16 predictors, the three clusters of changes in the index of the step test differ significantly from each other, as documented by the calculation of Mahalanobis distances (Table 4).

Table 4. Squared Mahalanobis Distances (above diagonal), F-values (df=17) and p-levels (below diagonal)

Clusters	PST_0	PST-	PST+
PST_0	0,00	8,86	3,85
PST-	3,64 $<10^{-3}$	0,00	12,46
PST+	2,80 0,002	6,23 $<10^{-6}$	0,00

The ultimate goal of discriminant analysis - predicting the nature of changes in the index of the step test - is realized using classifying functions (Table 5). 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 5. Coefficients and Constants for Classification Functions of Clusters

Clusters	PST+	PST_0	PST-
Predictors	p=,538	p=,287	p=,175
Popovych Step Test, points	-273,7	-265,4	-255,0
Sex Index, points	-115,4	-113,5	-107,8
$CD16^+$ NK Lymphocytes, %	15,93	15,92	16,59
Theophyl Resistant T Lymph., %	-16,13	-16,06	-16,37
Leukocytes total, G/L	47,71	48,33	48,62
Body Mass, % of norm	-2,64	-2,62	-2,48
Body Temperature Evening, ^0C	1774	1767	1768
Gench's Test, sec	-7,76	-7,69	-7,69
IgA Serum, g/L	-78,11	-77,16	-78,11
Trombocytes, G/L	,98	,98	1,01
Erythroc Sedimentat Rate, mm/h	7,08	7,14	6,59
Teslenko's Test, points	-9,54	-9,26	-8,94
MxDMn HRVas Vagal tone, sec	350,2	344,4	352,6
Eosinophiles, %	1,24	1,06	1,61
Microbial Count, Bact/Phagocyte	25,07	24,43	25,10
Pan Lymphocytes, %	17,99	17,96	18,19
Constants	-32500	-32257	-32367

As you can see (Table 6), the accuracy of the prediction of the decrease of the step test index is 92,9% (one error per 14 children), the increase of the index is assumed with an accuracy of 90,7% (4 errors per 43 children), and no changes - with accuracy 78,3% (5 errors per 23 children).

Table 6. Classification Matrix for Clusters
Rows: Observed classifications; Columns: Predicted classifications

Clusters	Percent correct	PST+ p=,538	PST_0 p=,287	PST- p=,175
PST+	90,7	**39**	**4**	0
PST_0	78,3	**3**	**18**	**2**
PST-	92,9	0	**1**	**13**
Total	87,5	42	23	15

DISCUSSION

First of all, it should be noted that the polyvariance of the changes of the step test index under the influence of balneofactors of the Truskavets' spa [34] is a separate manifestation of the polyvariance of balneoeffects on the functional state of the organism. Previously described various changes in the parameters of urination, cholekinetics, gastric and pancreatic secretion, hemodynamics, metabolism, hemostasis, immunity, autonomic and endocrine regulation in rats and humans [2-4,11-18,20,25-27,29-33]. As a rule, changes occur according to the law of the initial level and have a normalizing direction [2], characteristic of the action of adaptogens [11,25]. However, in some cases there is a further decline in reduced parameters or an increase in elevated [11,18,31], similar to individual immune responses to chronic stress [7]. We assume that this is due to the individual reactivity of the organism, which is determined by the state of the nervous, endocrine and immune systems, which interact closely [7,11,18,25]. It is shown that the main balneofactors of the Truskavets' spa (Naftussya water and Ozokerite) with their organic substances and autochthonous microflora [8,23-25,28] have a modulating effect on these main regulatory systems.

Unexpected prognostic ability of body temperature can be explained by its connection with the level of interleukin-1.

Another aspect of the discussion is the comparison of our results on the possibility of predicting the nature of the actotropic effects of balneofactors of Truskavets' spa with those obtained previously with the participation of one of the authors. In the observation of 42 adult patients of both sexes, in whom physical performance was assessed by the index of tachycardic-hypertensive response to veloergometric load, the accuracy of the prognosis of a positive actotropic effect was 81,0%; uncertain – 60,0%; negative – 72,7%, total correctness – 75,6%. Of the 72 initial signs taken into account, 10 were predictors: tachycardic-hypertensive reaction index, Klimov's atherogenic coefficient, cholesterol content in the composition of very low-density lipoproteins, sodium content in erythrocytes, activity of Na, K-ATPase of erythrocyte shadows, level of diastolic and systolic blood pressure, content of potassium and uric acid in plasma, as well as Ruzhylo-Popovych's index of contractile activity of myocardium [30].

In 18 rats, in order to predict the nature of the actotropic effect of 3-week Naftussya water intake, 6 initial signs were taken into account: dynamic physical performance - by the duration of swimming to exhaustion, static physical performance - by retention on a vertical pole, urine concentration of Na^+ and K^+, daily diuresis and body weight. The first 4 signs were prognostic. Even such scant information was sufficient to predict a significant increase in swimming duration in 33,3% of animals with an accuracy of 83,3%, a moderate increase in 44,4% - with an accuracy of 100%, and a reduction in swimming duration in 22,2% of rats - with an accuracy of 88,9%. The overall accuracy of the forecast was 88,9% [32].

Taken together, these and previous results indicate that the polyvariance of actotropic effects of balneofactors of Truskavets; spa is due to the individual reactivity of the organism and can be reliably predicted.

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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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