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METABOLIC PREDICTORS OF INDIVIDUAL IMMUNE RESPONSES TO ADAPTOGENIC BALNEOTHERAPY

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Summary

Background. Earlier four variants of the immune responses to adaptogenic balneotherapy have been identified. All four variants of immune responses are virtually unmistakably (with an accuracy of 97,7%) predicted by a set of 20 predictors including 12 immune blood parameters and one saliva parameter, 4 information parameters, 2 fecal microbiota parameters as well as erythrocyturia. In the next study discriminant analysis revealed that constellation 8 HRV and 5 Endocrine parameters as well as Gender of the patient predicts the nature of the immune response with an accuracy of 90,9%. The **purpose** of this study is to search for predictors of immune responses among the registered Metabolic parameters. **Material and methods.** The object of observation were 34 men and 10 women aged 24-70 years old, who came to the Truskavets' spa for the treatment of chronic pyelonephritis combined with cholecystitis in remission. We determined them the Electrokinetic index, Lipoproteines spectrum, plasma and daily urine levels of Electrolytes and Nitrous metabolites as well as basal and postprandial volume of gall-bladder. **Results.** Discriminant analysis revealed 16 parameters as the predictors. Of these, 3 reflect the level of Plasma Electrolytes, 5 - the Urine Electrolytes, another 2 - the levels of plasma and urine Urea. Other predictors were: Very Low Density Lipoprotein Cholesterol plasma level, Body Mass index, fasting gallbladder

volume and 30 minutes after cholekinetics, Electrokinetic index, and patient Age. These predictors, taken together, determine the nature of the immune response with an accuracy of 95,5%. **Conclusion**. The variety of immune responses to adaptogenic balneotherapy is quite strictly conditioned by the initial state of the neuroendocrine-immune complex, microbiota, cholekinetics and metabolism, as well as the age and sex of patients.

Key words: Electrolytes; Nitrous and Lipoproteins parameters; Cholekinetics; Immunity.

INTRODUCTION

Earlier four variants of the immune responses to adaptogenic balneotherapy have been identified [13,15]. All four variants of immune responses are virtually unmistakably (with an accuracy of 97,7%) predicted by a set of 20 predictors including 12 Immune blood parameters and one saliva parameter, 4 information parameters, 2 fecal Microbiota parameters as well as Erythrocyturia [10]. In the next study discriminant analysis revealed that constellation 8 HRV and 5 Endocrine parameters as well as Gender of the patient predicts the nature of the immune response with an accuracy of 90,9% [14]. The **purpose** of this study is to search for predictors of immune responses among the registered Metabolic parameters. This approach is in line with the functional-metabolic continuum [2].

MATERIAL AND METHODS

The object of observation were 34 men and 10 women aged 24-70 years old, who came to the Truskavets' spa for the treatment of chronic pyelonephritis combined with cholecystitis in remission.

At a receipt, we first determined them the rate of electronegative nuclei of buccal epithelium by intracellular microelectrophoresis on the device "Biotest" (Kharkiv State University), according to the method described [11]. The feasibility of this test is justified by previously obtained data on the relationship of Electrokinetic index with a number of functional and metabolic parameters of the body [6-8,12].

Then we estimated plasma Lipoproteines spectrum: total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of α-lipoproteins (by the enzyme method by Hiller G. [4] after precipitation of notα-lipoproteins); prae-β-lipoproteins (expected by the level of triacylglycerides, by a certain meta-periodate method); β-lipoproteins (expected by a difference between a total cholesterol and cholesterol in composition α-and prae-β-lipoproteins).

We determined also the plasma and daily urine levels of the Electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (flamming photometry); Nitrous metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method).

The analyzes were carried out according to the instructions described in the manual [1]. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a flamming spectrophotometer "СФ-47".

On the tone and motility of gall-bladder judged by its volume on an empty stomach in the morning and after 5, 15 and 30 min after ingestion cholekinetic (50 ml of 40% solution of xylitol). The method echoscopy (echocamera "Radmir") applicated [9].

Norms are borrowed from the database of the Truskavetsian Scientific School of Balneology.

Results processed by method of discriminant analyses [5], using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Following the accepted algorithm, the forward stepwise method revealed 16 parameters as the predictors. Of these, 3 reflect the level of **plasma electrolytes**, 5 - the **urine electrolytes**, another 2 - the levels of **plasma** and **urine** urea. Other predictors were: **very low density lipoprotein cholesterol plasma** level, **body mass** index, fasting **gallbladder** volume and 30 minutes after cholecinetics, **electrokinetic** index, and patient **age** (Table 1).

Next, the 16-dimensional space of discriminant variables transforms into 3-dimensional space of canonical roots. The canonical correlation coefficient is for Root 1 0,826 (Wilks' Λ=0,056; $\chi^2_{(48)}$=95; $p<10^{-4}$), for Root 2 0,821 (Wilks' Λ=0,176; $\chi^2_{(30)}$=57; p=0,002) and for Root 3 0,678 (Wilks' Λ=0,540; $\chi^2_{(14)}$=20; p=0,120). The first root contains 42,4% of discriminative properties, the second 40,8% and the minor 16,8% only.

Table 2 presents standardized and raw coefficients for discriminant variables which are used to the calculation of the discriminant root values for each person that enables their visualization in the information space of the roots.

Table 1. Discriminant Function Analysis Summary and Summary of Stepwise Analysis for Metabolic Predictors

Step 16, N of vars in model: 16; Grouping: 4 grps
Wilks' Lambda: 0,0559; approx. $F_{(49)}$=2,6; p<0,0001

Variables currently in the model	Wilks Λ	Partial Λ	F-remove (3,25)	p-level	Tolerancy	F to enter	p-level	Λ	F-value	p-level
Chloride Urine, mM/L	,079	,705	3,49	,031	,657	4,13	,012	,764	4,1	,012
Calcium Plasma, mM/L	,082	,682	3,88	,021	,517	3,84	,017	,589	3,9	,002
Magnesium Plasma, mM/L	,071	,792	2,19	,114	,793	3,21	,034	,470	3,7	10^{-3}
VLD LP Cholesterol, mM/L	,090	,620	5,12	,007	,366	2,11	,116	,402	3,4	10^{-3}
Calcium Excretion, mM/24h	,072	,772	2,46	,086	,073	2,79	,054	,326	3,3	10^{-3}
Phosphate Plasma, mM/L	,077	,724	3,18	,041	,494	1,91	,146	,280	3,1	10^{-4}
Electrokinetic Index, %	,058	,966	,30	,827	,154	2,40	,085	,231	3,1	10^{-4}
Body Mass Index, kg/m^2	,073	,769	2,50	,082	,527	2,35	,090	,190	3,1	10^{-4}
Urea Plasma, mM/L	,072	,779	2,37	,095	,708	2,69	,062	,152	3,2	10^{-4}
Potassium Excretio, mM/24h	,077	,724	3,18	,041	,245	1,72	,182	,130	3,1	10^{-4}
Urea Excretion, mM/24h	,073	,769	2,50	,082	,198	1,37	,272	,115	2,9	10^{-4}
Calcium Urine, mM/L	,067	,838	1,61	,213	,107	1,66	,198	,098	2,9	10^{-4}
Sodium Urine, mM/L	,066	,847	1,50	,238	,381	1,57	,219	,084	2,8	10^{-4}
GB Volume after 30 min, %	,077	,729	3,10	,045	,385	1,22	,323	,074	2,7	10^{-4}
Gallbladder Vol basal, mL	,068	,826	1,75	,182	,506	1,19	,333	,065	2,6	10^{-4}
Age, years	,065	,862	1,34	,285	,125	1,34	,285	,056	2,6	10^{-4}

Table 2. Standardized and Raw Coefficients and Constants for Metabolic Variables as Predictors

Coefficients	Standardized			Raw		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Chloride Urine, mM/L	-,654	,449	,218	-,0360	,0247	,0120
Calcium Plasma, mM/L	,431	,572	-,763	2,3341	3,0938	-4,1303
Magnesium Plasma, mM/L	-,578	,019	-,275	-14,38	,4797	-6,8352
VLD LP Cholesterol, mM/L	,849	-,391	,984	3,9175	-1,8047	4,5374
Calcium Excretion, mM/24h	,353	-1,178	2,137	,1494	-,4981	,9039
Phosphate Plasma, mM/L	-,014	,911	-,008	-,0761	4,8316	-,0412
Electrokinetic Index of Buccal Ep, %	,299	-,275	-,491	,0264	-,0243	-,0434
Body Mass Index, kg/m^2	,528	,603	,095	,1574	,1798	,0282
Urea Plasma, mM/L	,520	,385	,246	,4241	,3141	,2010
Potassium Excretion, mM/24h	-,939	,877	-,088	-,0227	,0212	-,0021
Urea Excretion, mM/24h	,658	-,150	-1,364	,0030	-,0007	-,0062
Calcium Urine, mM/L	,548	,712	-1,449	,5575	,7242	-1,473
Sodium Urine, mM/L	,471	-,294	,645	,0187	-,0116	,0256
Gallbladder Volume after 30min, %	,122	-,922	-,513	,0150	-,1133	-,0631
Gallbladder Volume basal, mL	-,260	,625	,275	-,0155	,0374	,0164
Age, years	,173	-1,223	-,417	,0133	-,0938	-,0320
		Constants		-4,810	-8,401	15,40
		Eigenvalues		2,149	2,067	,851
		Cumulative Prop		,424	,832	1,000

Table 3 shows the correlation coefficients of discriminant variables-predictors with canonical discriminant roots, the cluster centroids of roots, as well as the values of the discriminant variables-predictors.

Table 3. Correlations Variables-Canonical Roots, Means of Roots as well as Metabolic Variables as Predictors

	Correlations Variables-Roots			N/N (18)	N-/N (14)	S/S (10)	N/S (2)	Norm (30)	Cv
Root 1 (42,4%)	R 1	R 2	R 3	-1,61	+0,83	+1,16	+2,86		
Chloride Urine, mM/L	-,308	,198	,169	114	106	97,5	73	120	0,172
Magnesium Plasma, mM/L	-,325	-,095	-,072	0,859	0,819	0,824	0,817	0,900	0,056
Gallbladder Vol. basal, mL	-,206	,068	,082	55	52	43	33	46	0,230
GB Volume after 30 min, %	-,096	-,148	,007	67,5	68,1	65,8	60,9	62,0	0,081
Urea Excretion, mM/24h	,174	-,190	-,102	483	481	614	799	458	0,186
Calcium Excretion, mM/24h	,137	-,092	,006	3,71	4,03	4,90	5,50	4,38	0,214
Urea Plasma, mM/L	,160	-,003	,161	5,79	6,34	6,61	6,09	5,00	0,330
Calcium Urine, mM/L	,061	,039	-,047	2,33	2,45	2,29	2,57	3,12	0,214
Root 2 (40,8%)	R 1	R 2	R 3	-0,31	+1,83	-1,58	-2,10		
Calcium Plasma, mM/L	,175	,313	-,181	2,13	2,33	2,11	2,28	2,30	0,065
Phosphate Plasma, mM/L	,159	,305	,021	0,97	1,17	0,99	0,99	1,20	0,167
Body Mass Index, kg/m^2	,208	,225	,055	25,6	28,9	26,0	25,5	24,2	
Root 3 (16,8%)	R 1	R 2	R 3	-0,23	+0,04	+1,03	-3,34		
VLD LP Cholesterol, mM/L	,167	,034	,351	0,58	0,70	0,77	0,49	0,53	0,335
Potassium Excret, mM/24h	-,001	,073	,198	67	76	74	36	65	0,269
Sodium Urine, mM/L	-,085	,067	,189	123	122	121	95	161	0,211
Age, years	,055	,043	,129	48,0	51,4	51,4	45,0	49,7	0,256
Electrokinetic Index BE, %	-,018	,000	-,258	43,3	42,0	39,0	51,5	40,9	0,250

Figure 1 shows that the extreme left zone of the axis of the first root is occupied by members of the **N/N** cluster, while the opposite right is occupied by the members of the **N/S** cluster.

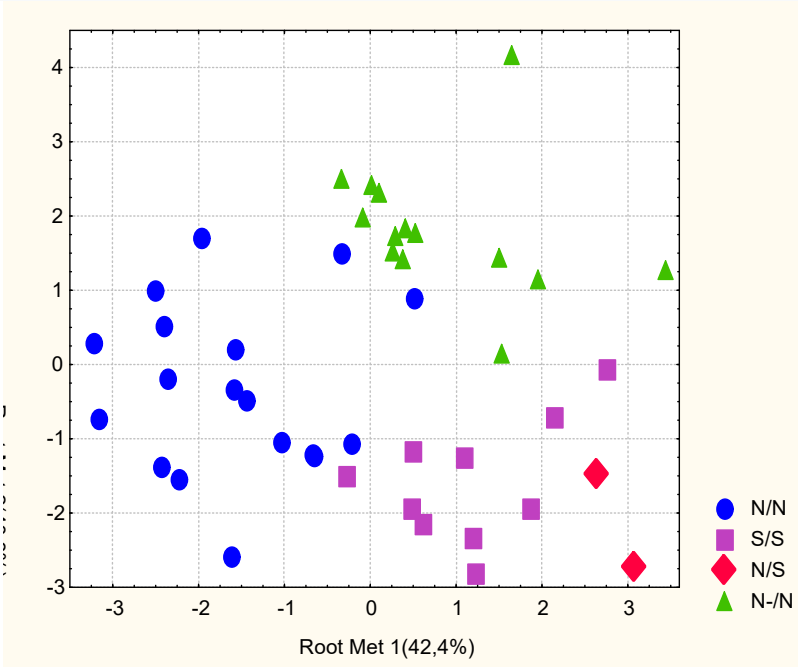

Fig. 1. Scatterplot of individual values of the first and second roots in which condensed information about initial values of metabolic parameters as predictors for the members of the four clusters

This reflects the **maximum** for sample the urinary concentration of chloride and plasma concentration of magnesium as well as the basal and postprandial gallbladder volumes, instead the **minimum** for sample values the calcium urinary excretion and concentration, urea excretion and plasma levels in the **N/N** cluster, on the one hand, and the **minimum**/**maximum** values of the listed predictors in the **N/S** cluster. The members of the other two clusters take an intermediate position and mix.

The members of the **N-/N** cluster delimit along the axis of the second root, occupying its upper zone, reflecting their maximum levels of calcium and phosphate plasma, as well as body mass index. Instead, the **S/S** and **N/S** clusters do not differ along this axis.

These clusters are clearly delineated along the axis of the third root (Fig. 2). The lower position of the **N/S** cluster members reflects their minimum levels of plasma prebeta-lipoprotein cholesterol, urinary sodium concentration and urinary excretion of potassium, as well as the minimum for sampling age. This is combined with the maximum electrokinetic index, which, by the way, is closely inversely correlated with age [11].

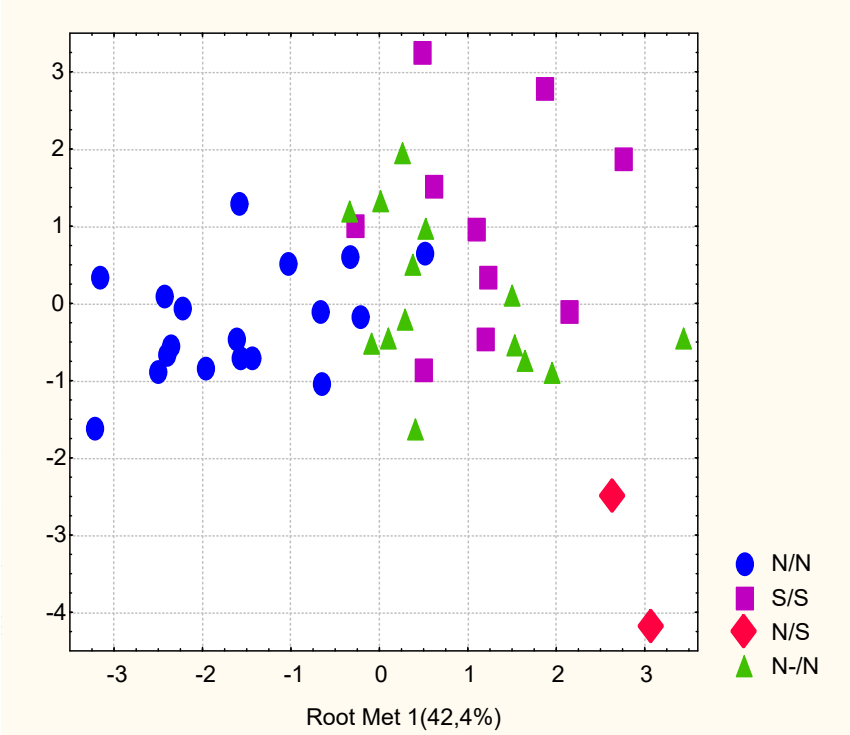

Fig. 2. Scatterplot of individual values of the first and third roots in which condensed information about initial values of metabolic parameters as predictors for the members of the four clusters

In general, all four clusters on the planes of the discriminant roots are quite satisfactorily delineated, which is documented by calculating the Mahalanobis distances (Table 4).

Table 4. Squared Mahalanobis Distances between Clusters, F-values (df=16,3) and p-levels

Clusters	N/N	S/S	N/S	N-/N
N/N	0	11,9	36,2	11,7
S/S	**2,75** 0,011	0,0	24,6	14,0
N/S	**1,33** 0,252	**0,86** 0,611	0,0	34,2
N-/N	**3,36** 0,003	**2,92** 0,008	**1,24** 0,307	0

The ultimate goal of discriminant analysis is realized with the help of classifying functions (Table 5).

Table 5. Coefficients and Constants for Classification Functions of Clusters

Clusters	N/N	S/S	N/S	N-/N
Variables	p=,409	p=,227	p=,045	p=,318
Chloride Urine, mM/L	,363	,248	,121	,332
Calcium Plasma, mM/L	142,1	139,4	159,8	153,3
Magnesium Plasma, mM/L	710,2	661,1	666,3	674,3
VLD LP Cholesterol, mM/L	2,228	21,10	8,850	9,135
Calcium Excretion, mM/24h	-5,952	-3,761	-7,205	-6,411
Phosphate Plasma, mM/L	42,80	36,40	33,94	52,97
Electrokinetic Index of Buccal Epit, %	4,594	4,643	4,890	4,594
Body Mass Index, kg/m^2	5,219	5,461	5,512	5,996
Urea Plasma, mM/L	12,41	13,44	13,12	14,18
Potassium Excretion, mM/24h	-,219	-,311	-,351	-,229
Urea Excretion, mM/24h	,071	,072	,105	,075
Calcium Urine, mM/L	12,60	11,36	18,38	15,12
Sodium Urine, mM/L	,103	,202	,127	,130
Gallbladder Volume after 30min, %	3,246	3,351	3,712	3,022
Gallbladder Volume basal, mL	-,812	-,882	-1,000	-,766
Age, years	2,501	2,616	2,828	2,323
Constants	-871,0	-855,8	-938,1	-897,5

As we can see (Table 6), three types of immune response to balneotherapy are predicted unmistakably, and **stable immunity** with two errors.

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

	Percent correct	N/N p=,409	S/S p=,227	N/S p=,045	N-/N p=,318
N/N	88,9	**16**	0	0	**2**
S/S	100	0	**10**	0	0
N/S	100	0	0	**2**	0
N-/N	100	0	0	0	**14**
Total	95,5	16	10	2	16

CONCLUSION

The variety of immune responses to adaptogenic balneotherapy is quite strictly conditioned by the initial state of the neuroendocrine-immune complex, microbiota, cholekinetics and metabolism, as well as the age and sex of patients.

The next article will analyze the links between changes in the parameters of immunity caused by adaptogenic balneotherapy on the one hand, and neuro-endocrine and metabolic parameters on the other.

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of

scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants. For all authors any conflict of interests is absent.

REFERENCES

1. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa: Astroprint; 1998: 608 p.
2. Gozhenko AI. Functional-metabolic continuum [in Russian]. J NAMS of Ukraine. 2016; 22(1): 3-8.
3. Gozhenko AI, Zukow W, Polovynko IS, Zajats LM, Yanchij RI, Portnichenko VI, Popovych IL. Individual Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment. RSW. UMK. Radom. Torun; 2019: 200 p.
4. Hiller G. Test for the quantitative determination of HDL cholesterol in EDTA plasma with Reflotron®. Klin Chem. 1987; 33: 895-898.
5. Klecka WR. Discriminant Analysis [trans. from English to Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika; 1989: 78-138.
6. Kyrylenko IG. Changes in electrokinetic index of buccal epithelium correlated with changes in some parameters of EEG, HRV, hemodynamics and metabolism. Experimental and Clinical Physiology and Biochemistry. 2018; 2(82): 5-14.
7. Kyrylenko IG, Fajda OI, Drach OV, Popel SL, Popel RL, Zukow W. Relationships between electrokinetic index of buccal epithelium and some functional and metabolic parameters at men with chronic pyelonephrite. Journal of Education, Health and Sport. 2016; 6(1): 302-314.
8. Kyrylenko IG, Flyunt I-SS, Fil' VM, Zukow W, Popovych IL. Changes in electrokinetic index of buccal epithelium correlated with changes in some parameters of immunity and fecal microbiocenosis. Journal of Education, Health and Sport. 2018; 8(10): 168-170.
9. Marfiyan OM, Korolyshyn TA, Barylyak LG, Kovbasnyuk MM, Yavors'kyi OV, Zukow W, Popovych IL. Neuroendocrine-immune and metabolic accompaniments of cholecystokinetic effects of balneotherapy on spa Truskavets'. Journal of Education, Health and Sport. 2015; 5(5): 21-30.
10. Mel'nyk OI, Struk ZD. Predictors of individual immune responses to adaptogens. Experimental and Clinical Physiology and Biochemistry. 2019; 88(4): 5-15.
11. Pat. 28113, Ukraine, NSI A61V10/00. Method of rapid testing efficiency rehabilitation of health. Shakhbazov VG, Kolupaeva TV, Shuvalov IM et al. 2000; Bul №5.
12. Shkorbatov YuG, Kolupayeva TV, Shakhbazov VG, Pustovoit PA. On the relationship between electrokinetic properties of human nucleated cells with some physiological parameters [in Russian]. Fiziologiya Cheloveka. 1995; 21(2): 25-27.
13. Struk ZD, Mel'nyk OI, Zukow W, Popovych IL. The diversity of immune reactions to balneotherapy and their accompaniments. Journal of Education, Health and Sport. 2019; 9(11): 349-373.
14. Struk ZD, Mel'nyk OI, Zukow W, Popovych IL. Vegetative and endocrine predictors of individual immune responses to adaptogenic balneotherapy. Journal of Education, Health and Sport. 2020; 10(1): 218-225.
15. Struk ZD, Mel'nyk OI, Mysakovets' OG. Individual immune responses to adaptogens and their predictors. In: Rehabilitation Medicine and Health-Resort Institutions Development. Proceedings of the 19th International Applied Research Conference (Kyïv, 11-12 December 2019). Edited by O. Gozhenko, W. Zukow. Toruń, Kyiv. 2019: 83-84.