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VEGETATIVE AND ENDOCRINE 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 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. The **purpose** of this study is to search for predictors of immune responses among the registered parameters of the autonomic nervous and endocrine systems. **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 HRV parameters and plasma levels of principal adaptation Hormones: Cortisol, Testosterone and Triiodothyronine. **Results.** 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%. **Conclusion.** The previously revealed variety of immune responses to adaptogenic balneotherapy is quite strictly conditioned by the initial state of the neuroendocrine-immune complex and microbiota as well as the sex of patients.

Key words: HRV, Cortisol, Testosterone and Triiodothyronine, Immunity.

INTRODUCTION

Earlier four variants of the immune responses to adaptogenic balneotherapy have been identified. In 40,9% of patients (N/N cluster), initially normal immune status (evaluated by 4 parameters of humoral immunity, 5 parameters of cellular immunity as well as 2 parameters of phagocytosis) did not change significantly (Figs. 1 and 2). In 31,8% of patients (N-/N cluster), the lower boundary level of immunity (due to the inhibition of Bactericidity of Neutrophils against *Staph. aureus* and *E. coli*) was completely normalized. In 22,7% of patients (S/S cluster) moderate suppression of Phagocytosis was reduced, but not up to normal. However, in 4,5% of people (N/S cluster), initially very increased level of Phagocytosis have been transformed into very decreased level in combination with a slight suppression of cellular immunity and slight activation of humoral immunity [14,15]. 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 [9]. Each type of immune response is characterized by a characteristic vegetative and endocrine support [10] along the lines of neuroendocrine-immune modulation [5,7,11,13,16,17].

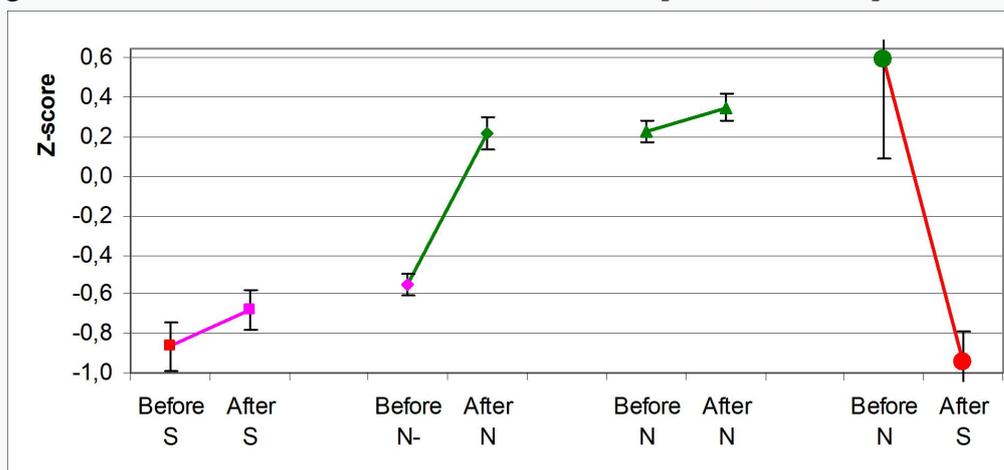


Fig. 1. Variants of immune status before and after balneotherapy

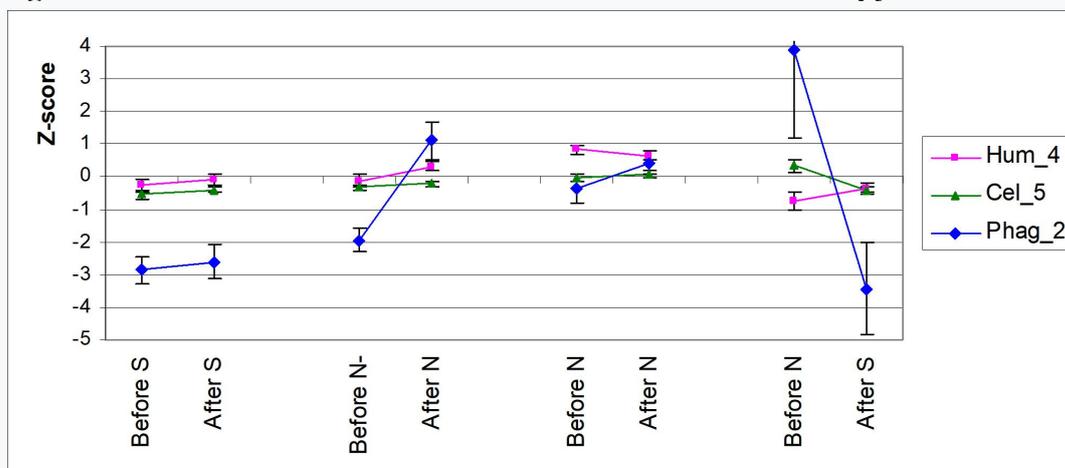


Fig. 2. Variants of the state of humoral and cellular immunity and phagocytosis before and after balneotherapy

The **purpose** of this study is to search for predictors of immune responses among the registered parameters of the autonomic nervous and endocrine systems.

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.

The state of the autonomic nervous system is estimated by parameters of heart rate variability (HRV). We recorded electrocardiogram in II lead (software and hardware complex "CardioLab+HRV" production "KhAI-MEDICA", Kharkiv). For further analysis the following parameters HRV were selected [1,3,6]. Temporal parameters (Time Domain Methods): 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 then 50 ms (pNN₅₀); heart rate (HR), the Mode (Mo), the Amplitude of Mode (AMo), variational sweep (MxDMn) as well as Triangulary Index (TINN). Spectral parameters (Frequency Domain Methods): spectral power (SP) 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 ultra low-frequency (ULF, range 0,015÷0,003 Hz). On the basis of these parameters were calculated proportion of SP bands (% of Total Power) and classical indexes: LF/HF, LFnu=100%•LF/(LF+HF), Centralization Index=(VLF+LF)/HF; Baevskiy's Stress Index (BSI=AMo/2•Mo•MxDMn) and Baevskiy's Activity Regulatory Systems Index (BARS) [1] as well as the Entropy (h) of HRV [12].

To assess endocrine status in the morning on an empty stomach we determined plasma levels of principal adaptation Hormones: Cortisol, Testosterone and Triiodothyronine (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Алкор Био", XEMA Co, Ltd and DRG International Inc).

We determined also the plasma and daily urine levels of the electrolytes: calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (flaming photometry). The analyzes were carried out according to the instructions described in the manual [4]. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a flaming spectrophotometer "CФ-47".

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients (Cap/Pp)^{0.5} and (Pu/Cau)^{0.5}, calcitonin by coefficients (Cap•Pp)^{-0.5} and (Cau•Pu)^{0.5} as well as mineralocorticoid by coefficients (Nap/Kp)^{0.5} and (Ku/Nau)^{0.5}, based on their classical effects and recommendations by IL Popovych [5].

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

Results processed by method of discriminant analyses [8], using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Following the accepted algorithm, the forward stepwise method revealed 8 **HRV** parameters and 5 **endocrine** parameters as the predictors, as well as the sex of the patient, quantified as Sex Index (men rated one point, women two points) (Table 1.). The latter predictor is closely related to testosterone levels, normalized by sex and age (see Figure 1 from [10]).

Table 1. Discriminant Function Analysis Summary and Summary of Stepwise Analysis
 Step 14, N of vars in model: 14; Grouping: 4 grps
 Wilks' Lambda: 0,111; approx. $F_{(43)}=2,1$; $p=0,002$

Variables currently in the model	Wilks Λ	Partial Λ	F-remove (3,27)	p-level	Tole-ran-cy	F to enter	p-level	Λ	F-value	p-level
(Cap•Pp)^{0.5} as Calcitonin	,191	,581	6,50	,002	,30	5,58	,003	,705	5,6	,0027
Sex Index (M=1; F=2), ps	,142	,783	2,49	,081	,55	3,57	,023	,553	4,5	,0006
(Ku/Nau)^{0.5} as Mineraloc	,161	,690	4,05	,017	,67	2,39	,083	,465	3,8	,0004
Testosterone normalized	,152	,730	3,34	,034	,61	2,39	,084	,390	3,5	,0003
Cortisol Plasma	,148	,752	2,97	,050	,48	2,58	,068	,321	3,4	,0001
HF HRV normalized	,131	,851	1,57	,219	,35	1,47	,238	,285	3,1	,0002
Baevskiy's ARS Index	,123	,904	,96	,428	,43	1,38	,267	,254	2,9	,0003
(Cap/Pp)^{0.5} as Parathyroid	,144	,769	2,70	,066	,31	1,16	,341	,230	2,7	,0004
ULF HRV, msec²	,134	,830	1,85	,162	,12	1,15	,345	,207	2,5	,0007
VLF HRV, %	,139	,800	2,25	,106	,15	1,27	,301	,185	2,4	,0009
ULF HRV, %	,124	,893	1,08	,375	,14	1,18	,334	,165	2,3	,0012
SDNN HRV, msec	,137	,808	2,14	,119	,03	1,27	,302	,146	2,2	,0015
Triangular Index HRV	,132	,840	1,71	,188	,14	1,62	,208	,124	2,2	,0014
VLF HRV, msec²	,124	,893	1,08	,374	,03	1,08	,374	,111	2,1	,0020

Next, the 14-dimensional space of discriminant variables transforms into 3-dimensional space of canonical roots, which are a linear combination of discriminant variables. The canonical correlation coefficient is for Root 1 0,805 (Wilks' $\Lambda=0,111$; $\chi^2_{(42)}=75$; $p=0,001$), for Root 2 0,740 (Wilks' $\Lambda=0,316$; $\chi^2_{(26)}=39$; $p=0,047$) and for Root 3 0,550 (Wilks' $\Lambda=0,698$; $\chi^2_{(12)}=12$; $p=0,427$). The major root contains 52,8% of discriminative properties, the second 34,8% and the minor 12,4% only.

Table 2 presents standardized and raw 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.

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

Variables	Coefficients			Standardized			Raw		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
(Cap•Pp)^{0.5} as Calcitonin Act ivity	1,441	,167	,373	21,44	2,493	5,545			
Sex Index (M=1; F=2), points	-,046	,844	,069	-,116	2,152	,177			
(Ku/Nau)^{0.5} as Mineralocorticoid activity	-,569	,526	,572	-6,415	5,936	6,448			
Testosterone normalized by sex&age, Z	,411	-,709	,445	,250	-,431	,270			
Cortisol Plasma, nM/L	-,034	-,968	-,130	-,0001	-,0038	-,0005			
HF HRV normalized by age, Z	,802	,054	,050	,358	,024	,022			
Baevskiy's Activity Regulatory Systems Ind	-,141	,207	,789	-,0539	,0790	,3010			
(Cap/Pp)^{0.5} as Parathyroid Activity	-1,032	,321	-,163	-7,619	2,368	-1,200			
ULF HRV, msec²	-,953	1,271	,041	-,0109	,0146	,0005			
VLF HRV, %	1,437	,160	,088	,0876	,0098	,0053			
ULF HRV, %	,620	-,899	,465	,150	-,217	,112			
SDNN HRV, msec	3,381	,207	,256	,181	,011	,014			
Triangular Index HRV, units	-1,190	-,357	,802	-,298	-,089	,201			
VLF HRV, msec²	-2,118	-,383	-1,309	-,0023	-,0004	-,0014			
			Constants	-6,393	-8,381	-8,705			
			Eigenvalues	1,841	1,211	,433			
			Cumulative Prop.	,528	,876	1,000			

Table 3. Correlations Variables-Canonical Roots, Means of Roots as well as HRV and Endocrine Variables as Predictors

Variables	Correlations Variables-Roots			N-/N (14)	N/N (18)	S/S (10)	N/S (2)	Norm (88)	Cv
	R1	R2	R3						
Root 1 (52,8 %)	R1	R2	R3	-1,81	+0,47	+1,40	+1,48		
(Cap•Pp) ^{0.5} as Calcitonin Act	,435	,232	,096	0,61	0,70	0,67	0,70	0,60	0,167
SDNN HRV, msec	,188	,180	-,001	38,4	53,5	45,5	45,5	55	0,201
(Cap/Pp) ^{0.5} as Parathyroid Act	,168	,092	-,128	1,42	1,50	1,48	1,52	1,38	0,167
Triangulary Index HRV, units	,100	,137	,079	9,8	11,7	11,1	10,0	11,2	0,217
Baevskiy's ARS Index, points	-,153	-,055	,256	4,31	2,94	3,50	1,50	1,50	0,624
Root 2 (34,8%)	R1	R2	R3	-0,45	+1,18	-1,05	-2,19		
Sex Index (M=1; F=2), points	,004	,448	-,161	1,14	1,44	1	1	1,50	
VLF HRV, msec ²	,101	,215	-,148	924	1542	1014	1271	1384	0,578
ULF HRV, msec ²	-,024	,193	,129	72	94	59	8	122	1,021
Cortisol Plasma, nM/L	-,206	-,183	-,212	718	528	542	762	405	0,315
Root 3 (12,4%)	R1	R2	R3	+0,05	-0,15	+0,69	-2,45		
(Ku/Nau) ^{0.5} as Mineralocort ac	-,203	,191	,399	0,59	0,57	0,54	0,41	0,54	0,269
Testosterone normalized, Z	,211	-,246	,348	+1,07	+1,09	+2,80	+1,49	0	
Males (n=12+10+10+2)				+1,22	+1,20	+2,80	+1,49	0	0,407
Females (n= 2+ 8+ 0+0)				+0,18	+0,96	lack	lack	0	0,600
ULF HRV, %	-,033	,057	,224	3,58	3,46	3,63	0,38	4,3	0,926
HF HRV normalized by age, Z	,171	,093	,211	-0,02	+0,42	+0,64	-0,35	0	
VLF HRV, %	-,088	,042	-,393	54,8	51,7	41,5	62,7	53,4	0,378

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.

Localization of members of the N-/N cluster in the extreme left zone of the axis of the first root (Fig. 3) reflects their minimum for the sample the levels of calcitonin and parathyroid activity, which are quite average. However, the minimum for the sample markers for overall rhythm variability (SDNN and TINN) are below average. Instead, Baevskiy's Activity Regulatory Systems Index is maximum for the sample and displays a moderate strain.

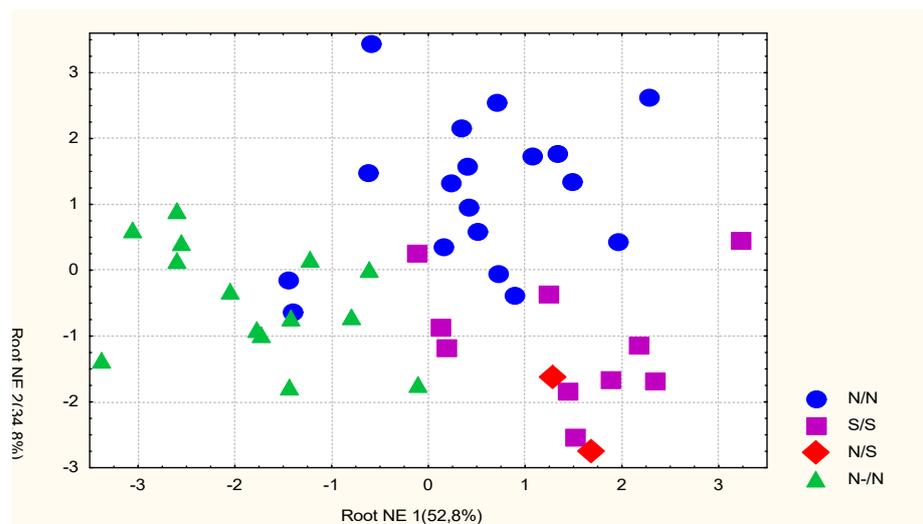


Fig. 3. Scatterplot of individual values of the first and second roots in which condensed information about initial values of the HRV and endocrine parameters as predictors for the members of the four clusters

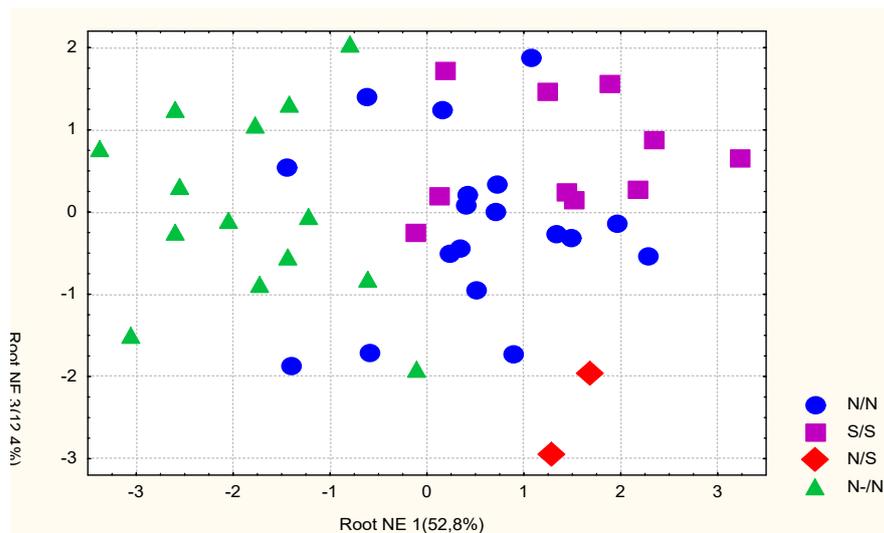


Fig. 4. Scatterplot of individual values of the first and third roots in which condensed information about initial values of the HRV and endocrine parameters as predictors for the members of the four clusters

Members of other clusters occupy the right zone of the axis and mix, reflecting the absence of significant inter-cluster differences between the levels of the mentioned parameters, which are higher/lower than those in the previous cluster. The members of the **N/N** cluster are separated from the others along the axis of the second root by occupying its upper zone. This reflects, first and foremost, the highest sex index for the sample, indicating a nearly equal proportion of men and women (10/8), while the other clusters are wholly or almost entirely male. Other characteristic features of this cluster are the maximum absolute spectral power of VLF and ULF bands of HRV in combination with the minimum plasma cortisol level for sampling. Finally, the last two clusters delineate along the axis of the third root (Fig. 4). Higher localization of members of the **S/S** cluster relative to the **N/S** cluster reflects their higher levels of mineralocorticoid activity, vagus tone, testosterone normalized by sex and age, and the relative spectral power of the ULF band HRV combined with a lower level of such VLF band.

Previously, we showed [2] that the absolute power of the VLF band correlate significantly with the markers of the vagus tone directly, and with the markers of sympathetic tone inversely. This gives us reason to believe that VLF band is vagus marker. Instead, the relative power of VLF band is associated with vagus and sympathetic markers in the opposite way, that is, a sympathetic marker, as is commonly recognized for LFnu. The physiological interpretation of ULF band HRV is still unknown to us.

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=14,3) and p

Clusters	N/N	S/S	N/S	N-/N
N/N	0	7,2	19,4	8,6
S/S	2,05 0,054	0	12,3	12,2
N/S	0,89 0,582	0,54 0,890	0	22,1
N-/N	3,07 0,006	3,12 0,005	0,99 0,488	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
(Cap•Pp)^{0.5} as Calcitonin Activity	274,1	293,2	274,7	222,3
Sex Index (M=1; F=2), points	29,06	24,30	21,30	25,86
(Ku/Nau)^{0.5} as Mineralocorticoid activity	130,4	116,6	89,03	136,6
Testosterone normalized by sex&age, Z	1,420	2,841	2,498	1,605
Cortisol Plasma, nM/L	-,004	,004	,010	,003
HF HRV normalized by age, Z	-,983	-,685	-,752	-1,833
Baevskiy's Activity Regulatory Systems Index	6,620	6,647	5,604	6,673
(Cap/Pp)^{0.5} as Parathyroid Activity	20,66	7,261	7,726	33,90
ULF HRV, msec²	,091	,048	,029	,092
VLF HRV, %	2,094	2,159	2,138	1,880
ULF HRV, %	-,025	,695	,599	,010
SDNN HRV, msec	4,141	4,297	4,256	3,713
Triangular Index HRV, units	-,203	-,112	-,670	,660
VLF HRV, msec²	-,089	-,091	-,086	-,083
Constants	-279,3	-275,4	-245,4	-254,0

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.

As we can see (Table 6), the adverse immune response of both members of the **N/S** cluster is retrospectively predicted by the set of vegetative and endocrine predictors unmistakably, which attests to its regularity despite the critical number of cluster members. The other two types of immune response to balneotherapy are predicted with single errors and **stable immunity** with two errors.

Table 6. Classification Matrix

Rows: Observed classifications; Columns: Predicted classifications

	Percent correct	N/N	S/S	N/S	N-/N
		p=,409	p=,227	p=,045	p=,318
N/N	88,9	16	0	0	2
S/S	90,0	1	9	0	0
N/S	100	0	0	2	0
N-/N	92,9	0	0	1	13
Total	90,9	17	9	3	15

The next article will be devoted to the search for metabolic predictors of the variety of immune responses to balneotherapy.

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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.

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