KOZYAVKINA, Nataliya, VOVCHYNA, Yuliya, VORONYCH-SEMCHENKO, Nataliya, ZUKOW, Walery, POPOVYCH, Dariya & POPOVYCH, Igor. Immune accompaniment of quantitative-qualitative blood pressure clusters in patients of Truskavets' spa. Journal of Education, Health and Sport. 2022;12(3):320-329. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2022.12.03.028
https://apcz.umk.pl/JEHS/article/view/43970
https://zenodo.org/record/7931575

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343.
Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).

Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159.
Przypisane dyscypliny naukowe:Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 10.02.2022. Revised: 28.02.2022. Accepted: 26.03.2022.

Immune accompaniment of quantitative-qualitative blood pressure clusters in patients of Truskavets' spa

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Abstract

Background. Earlier we studied the neural, endocrine, and metabolic accompaniments of quantitative-qualitative blood pressure (BP) clusters of profile patients of Truskavets' spa. The **purpose** of this study is to clarify the immune accompaniment in the same contingent. **Materials and methods.** Under an observations were 44 patients with chronic pyelonephritis and cholecystitis in the phase of remission. Testing was performed twice - on admission and after 7-10 days of standard balneotherapy. The main object of the study was BP. We determined in the blood the relative content of leukocyte forms, of T-lymphocytes and their killer, helper and regulatory subpopulations as well as NK- and B-lymphocytes; in serum - the concentration of C-reactive protein, Tumor Necrosis Factor-α, Interleukins 1β and 6, immunoglobulins classes G, A, and M as well as circulating immune complexes; in saliva - IgG, IgA, and secretory IgA. In addition, we determined parameters of phagocytosis by neutrophils of Staph. aureus and E. coli; components of stool and urine microbiota. **Results**. The forward stepwise program identified 18 parameters as characteristic of quantitative-qualitative blood pressure clusters. In addition to BP parameters by default, the most informative among them are serum levels of IL-6 and TNF-α as well as activity and intensity of phagocytosis by neutrophils of Staph. aureus. The accuracy of patient classification is 96,6%. **Conclusion**. The quantitative-qualitative blood pressure clusters have a characteristic immune accompaniment.

Keywords: blood pressure, immunity, discriminant analysis, Truskavets' spa.

INTRODUCTION

Earlier we showed that profile patients of Truskavets' spa are characterized by a wide range of blood pressure - from low norm to arterial hypertension III - that correspond to the hemodynamics parameters [6]. Than we clarified the neural and endocrine [7,8] as well as metabolic [9] accompaniments of quantitative-qualitative blood pressure clusters in the same contingent.

The **purpose** of this study is to clarify the immune accompaniments of quantitative-qualitative blood pressure clusters in the same contingent.

MATERIALS AND METHODS

Under an observations were 34 males and 10 females by age 24-76 years with chronic pyelonephritis and cholecystitis in the phase of remission. Testing was performed twice - on admission and after 7-10 days of standard balneotherapy (drinking of bioactive water Naftussya, applications of ozokerite, mineral pools).

The main object of the study was blood pressure (BP). Systolic and diastolic BP was measured (by tonometer "Omron M4-I", Netherlands) in a sitting position three times in a row.

Retrospectively, 5 quantitative-qualitative blood pressure clusters were created (Fig. 1) according to the existing gradation [2,13,20].

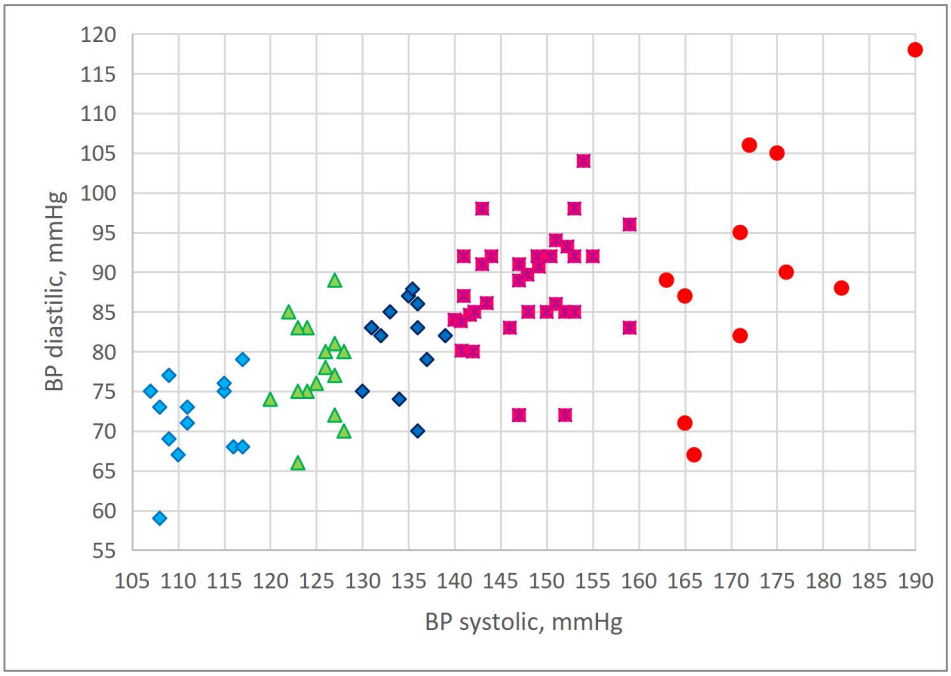

Fig. 1. Diagram of scattering of systolic and diastolic blood pressure of patients of Truskavets' spa

Immune status evaluated as described in the manuals [4,12]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD25, CD22 and CD56 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Immunoglobulins of classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method) as well as C-

reactive protein (by the ELISA with the use of analyzer “RT-2100C”), Tumor Necrosis Factor-α, Interleukins 1β and 6 (ELISA, analyzer “Stat Fax 303”, USA, reagents from “Vector-Best”, RF).

Based on the normalized values of humoral (5 parameters) and cellular (4 parameters) links, the integral index of immunity was calculated as the average of nine Z-scores.

The set of immune parameters of saliva was IgG, IgA, and secretory IgA (ELISA, analyser “Immunochem”, USA).

In portion of the capillary blood we counted up Leukocytogram and calculated the Entropy (h) of Leukocytogram (LCG) as well as its Strain Index using IL Popovych’s equations [3,17]:

$$hLCG = - [L \bullet \log_2 L + M \bullet \log_2 M + E \bullet \log_2 E + SNN \bullet \log_2 SNN + StubN \bullet \log_2 StubN]/\log_2 5;$$

$$\text{Strain Index-1} = [(Eos/3,5-1)^2 + (StubN/3,5-1)^2 + (Mon/5,5-1)^2 + (Leuk/6-1)^2]/4.$$

Parameters of phagocytic function of neutrophils estimated as described by MM Kovbasnyuk [11,18]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Take into account the following parameters of Phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger’s Phagocytic Index PhI), intensity (number of microbes absorbed one phagocytes - Microbial Count MC or Right’s Index) and completeness (percentage of dead microbes - Killing Index KI). On the basis of the registered partial parameters of phagocytosis, taking into account the content of neutrophils (N) in 1 L of blood, the integral parameter - the bactericidal capacity of neutrophils - was calculated by the equation:

$$BCCN\ (10^9\ Bact/L) = N\ (10^9/L) \bullet PhI\ (\%) \bullet MC\ (Bact/Phag) \bullet KI\ (\%) \bullet 10^{-4}.$$

The condition of microbiota is evaluated on the results of sowing of feces. The levels of bacteriuria, leukocyturia, and erythrocyturia were also assessed by routine methods.

Reference values of variables are taken from the database of the Truskavetsian Scientific School of Balneology [17].

For statistical analysis used the software package "Statistica 6.4".

RESULTS

In order to identify among the registered parameters, those for which the blood pressure clusters differ from each other, a discriminant analysis was performed [5]. The program forward stepwise included in the discriminant model 18 parameters. In addition to BP parameters by default, the following variables were identified as characteristic: 2 proinflammatory cytokines, 6 immune parameters of blood and saliva, 5 parameters of phagocytosis, 2 markers of pyelonephritis as well as strain index of leukocytogram. A number of parameters that were found to be outside the discriminant model are also worthy of attention (Tables 1 and 2).

Table 1. Discriminant Function Analysis Summary for Immune Variables, their actual levels (Mean±SE) for Clusters of Blood Pressure as well as Reference levels and Coefficients of Variability

Step 18, N of vars in model: 18; Grouping: 5 grs; Wilks' Λ: 0,0155; approx. $F_{(72)}$=6,85; $p<10^{-6}$

Variables currently in the model	Clusters of Blood Pressure (n)					Parameters of Wilk's Statistics						
	AH II (11)	AH I (35)	High N (13)	No-rm (16)	Low N (13)	Wilks' Λ	Par-tial Λ	F-re-move (4,66)	p-level	Tole-rancy	Refe-rence (88)	Cv
BP Systolic, mmHg	172 2,5	148 0,9	134 0,8	125 0,6	112 1,0	0,166	0,094	159	10^{-6}	0,658	124,5 1,6	,122
BP Diasto-lic, mHg	90,7 4,5	87,6 1,2	81,3 1,5	77,8 1,5	71,5 1,5	0,017	0,911	1,61	0,181	0,697	79,0 0,7	,086
Interleukin-6, ng/L	7,22 0,76	4,62 0,55	4,76 0,91	3,64 0,81	4,61 0,88	0,019	0,802	4,08	0,005	0,368	4,25 0,15	,324
TNF-α, ng/L	6,94 0,42	6,06 0,25	6,29 0,60	5,15 0,46	5,97 0,33	0,019	0,839	3,17	0,019	0,355	4,90 0,17	,326
Immunity Integral Ind	0,24 0,13	-0,15 0,08	0,10 0,12	0,01 0,13	0,24 0,15	0,020	0,788	4,43	0,003	0,276	0	
CIC, units	42 6	34 2	30 3	34 4	43 5	0,017	0,926	1,32	0,270	0,784	45 2	,389
IgA Serum, g/L	2,03 0,05	1,58 0,09	1,91 0,14	1,78 0,11	1,92 0,12	0,017	0,906	1,71	0,158	0,404	1,875 0,03	,167
Secret IgA Saliva, g/L	505 20	491 9	464 20	495 17	504 13	0,018	0,882	2,21	0,077	0,400	622 10	,153
IgA Saliva, g/L	149 9	123 5	136 10	133 10	156 12	0,017	0,889	2,05	0,097	0,328	163 4	,241
Monocytes, %	5,05 0,48	6,55 0,36	6,39 0,55	5,38 0,49	5,15 0,61	0,016	0,951	0,85	0,500	0,614	6,00 0,05	,083
Rod shaped Neutrop, %	2,85 0,38	2,40 0,17	3,10 0,36	2,64 0,27	2,80 0,25	0,017	0,904	1,75	0,150	0,497	4,25 0,07	,147
Popovych's Strain Ind-1	0,11 0,02	0,18 0,02	0,14 0,02	0,16 0,03	0,11 0,02	0,017	0,936	1,13	0,351	0,613	0,10 0,01	,559
Phag Ind vs St. aur., %	98,5 0,34	98,62 0,24	99,34 0,21	99,2 0,19	98,91 0,32	0,018	0,883	2,19	0,080	0,370	98,3 0,19	,018
Killing vs St. aur., %	46,1 1,5	49,6 1,4	53,8 3,0	47,3 2,3	50,4 1,8	0,018	0,856	2,78	0,034	0,348	58,9 0,9	,142
Mic Cou St. aur., B/Ph	59,7 1,9	61,3 1,6	63,2 1,9	64,1 1,9	63,9 1,9	0,017	0,929	1,27	0,292	0,398	61,6 1,1	,160
Phagoc Ind vs E. coli,%	99,2 0,18	98,62 0,26	99,61 0,18	98,9 0,47	99,38 0,18	0,017	0,929	1,27	0,291	0,336	98,3 0,13	,012
Leukocytu-ria, lgLeu/L	3,75 0,16	3,35 0,10	3,12 0,23	3,35 0,18	3,28 0,19	0,018	0,860	2,69	0,038	0,429	3,0 0,02	,070
Erhytrocyt-uria, lgEr/L	2,99 0,10	3,07 0,04	3,05 0,07	3,06 0,11	3,02 0,09	0,017	0,939	1,08	0,376	0,691	2,7 0,02	,078
Variables currently't in model	AH II (11)	AH I (35)	High N (13)	No-rm (16)	Low N (13)	Wilks' Λ	Par-tial Λ	F to enter	p-level	Tole-rancy	Refe-rence (88)	Cv
C-Reactive Prot., µg/L	2,92 0,18	2,54 0,11	2,64 0,26	2,14 0,20	2,50 0,15	0,015	0,956	0,752	0,560	0,274	2,18 0,08	,324
$CD4^+CD25^+$ T-regul., %	18,8 1,1	21,1 0,6	19,1 1,1	20,8 1,2	17,6 1,3	0,015	0,977	0,378	0,823	0,386	16,4 0,3	,153
$CD4^+$ T-hel-per Lym, %	33,7 1,9	27,4 1,0	33,1 1,9	30,5 2,2	35,9 2,6	0,015	0,968	0,544	0,704	0,052	39,5 0,7	,164
Bifidobacte-ria,lgCFU/g	5,35 0,30	5,69 0,19	5,78 0,24	5,52 0,36	5,38 0,35	0,015	0,978	0,367	0,832	0,468	6,94 0,01	,011
Lactobacilli lgCFU/g	6,00 0,38	6,48 0,23	6,69 0,26	6,22 0,45	6,09 0,45	0,015	0,978	0,368	0,831	0,432	8,10 0,01	,015
E. coli com., lgCFU/g	8,16 0,06	8,30 0,04	8,33 0,07	8,26 0,07	5,24 0,09	0,015	0,987	0,206	0,934	0,415	8,66 0,04	,045

Table 2. Summary of Stepwise Analysis for Variables, ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-value
BP Systolic, mmHg	298	10^{-6}	0,065	298	10^{-6}
Immunity Integral Index	3,83	0,007	0,055	67,0	10^{-6}
Monocytes, %	2,13	0,084	0,050	37,8	10^{-6}
Interleukin-6, ng/L	2,52	0,047	0,044	27,2	10^{-6}
Leukocyturia, lgLeu/L	1,49	0,213	0,041	21,3	10^{-6}
Circulating Immune Complex, units	1,65	0,170	0,038	17,7	10^{-6}
Tumor Necrosis Factor-α, ng/L	1,35	0,260	0,035	15,2	10^{-6}
BP Diastolic, mHg	1,59	0,186	0,033	13,5	10^{-6}
Rod shaped Neutrophils, %	1,61	0,180	0,030	12,2	10^{-6}
Killing Index vs Staph. aureus, %	1,47	0,220	0,028	11,1	10^{-6}
IgA Serum, g/L	1,48	0,217	0,026	10,2	10^{-6}
Phagocytose Index vs St. aureus, %	1,49	0,213	0,024	9,55	10^{-6}
Secretory IgA Saliva, g/L	1,54	0,200	0,022	8,97	10^{-6}
IgA Saliva, g/L	1,44	0,229	0,020	8,47	10^{-6}
Popovych's Strain Index-1	1,28	0,288	0,019	8,00	10^{-6}
Microbian Count for St. aureus, B/Ph	1,09	0,367	0,018	7,57	10^{-6}
Phagocytose Index vs E. coli, %	1,13	0,351	0,017	7,19	10^{-6}
Erhytrocyturia, lgEr/L	1,08	0,376	0,016	6,85	10^{-6}

Next, the 18-dimensional space of discriminant variables transforms into 4-dimensional space of a canonical roots. For Root 1 r*=0,976 (Wilks' Λ=0,0155; $\chi^2_{(72)}$=314; p<10^{-6}), for Root 2 r*=0,637 (Wilks' Λ=0,330; $\chi^2_{(51)}$=84; p=0,003), for Root 3 r*=0,572 (Wilks' Λ=0,556; $\chi^2_{(32)}$=44; p=0,072), and for Root 4 r*=0,416 (Wilks' Λ=0,827; $\chi^2_{(15)}$=14; p=0,498). The first root contains 93,6% of discriminative opportunities, the second 3,2%, the third 2,2%, the last 1,0% only, therefore will be ignored in the future.

Table 3 presents raw and standardized coefficients for discriminant variables, which are used for the calculation of the discriminant root values for each person, which enables the visualization of each patient in the information space of the roots (Figs. 2 and 3).

Table 3. Standardized and Raw Coefficients and Constants for Variables

Coefficients	Standardized			Raw		
Variables currently in the model	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
BP Systolic, mmHg	-1,202	-0,003	-0,049	-0,248	-0,001	-0,010
Immunity Integral Index	-0,363	-0,817	0,860	-0,675	-1,518	1,599
Monocytes, %	0,113	0,339	-0,223	0,055	0,166	-0,109
Interleukin-6, ng/L	-0,481	-0,796	0,245	-0,192	-0,318	0,098
Leukocyturia, lgLeu/L	-0,578	-0,043	-0,133	-0,878	-0,065	-0,203
Circulating Immune Complex, units	0,022	-0,250	-0,059	0,001	-0,016	-0,004
Tumor Necrosis Factor-α, ng/L	0,354	0,583	-0,683	0,218	0,359	-0,421
BP Diastolic, mHg	0,299	0,319	-0,022	0,038	0,040	-0,003
Rod shaped Neutrophils, %	0,198	0,498	-0,386	0,181	0,457	-0,354
Killing Index vs Staph. aureus, %	-0,097	0,414	-0,992	-0,012	0,049	-0,117
IgA Serum, g/L	0,260	0,078	-0,711	0,566	0,169	-1,547
Phagocytose Index vs St. aureus, %	-0,279	0,439	0,445	-0,241	0,379	0,384
Secretory IgA Saliva, g/L	0,0004	-0,291	0,883	0,00001	-0,005	0,015
IgA Saliva, g/L	0,113	-0,348	-0,803	0,003	-0,010	-0,024
Popovych's Strain Index-1	0,141	0,402	0,208	1,293	3,682	1,901
Microbian Count for St. aureus, B/Ph	0,199	0,100	-0,597	0,024	0,012	-0,073
Phagocytose Index vs E. coli, %	0,247	0,245	-0,614	0,184	0,183	-0,458
Erythrocyturia, lgEr/L	0,303	-0,050	-0,005	0,977	-0,162	-0,015
			Constants	32,830	-61,01	22,48
			Eigenvalues	20,25	0,683	0,487
			Cumulative proportions	0,936	0,968	0,990

Table 4 shows the correlation coefficients of blood pressure and immune parameters with canonical discriminant roots; the cluster centroids of roots; and Z-scores of the variables.

Table 4. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Blood Pressure and Immune Variables

Variables currently in the model	Correlations Variables-Roots			AH II (11)	AH I (35)	High N (13)	Norm (16)	Low N (13)
Root 1 (93,6%)	**R 1**	**R 2**	**R 3**	**-8,5**	-1,8	+1,5	+3,3	**+6,6**
BP Systolic	**-0,840**	0,254	-0,064	**+3,15**	+1,54	+0,64	+0,04	**-0,84**
BP Diastolic	**-0,174**	0,320	0,087	**+1,79**	+1,31	+0,35	-0,19	**-1,14**
Interleukin-6	**-0,063**	-0,193	-0,204	**+2,16**	+0,27	+0,37	-0,44	+0,26
Tumor Necrosis Factor-α	**-0,049**	-0,063	-0,307	**+1,28**	+0,73	+0,87	+0,15	+0,67
C-Reactive Protein				**+1,05**	+0,57	+0,65	-0,05	+0,45
Phagocytose Index vs St. aureus	**0,040**	0,024	-0,070	**+0,14**	+0,18	+0,59	+0,53	+0,35
Microbial Count for Staph. aur.	**0,041**	-0,008	0,012	**-0,19**	-0,03	+0,16	+0,26	+0,23
Root 2 (3,2%)	**R 1**	**R 2**	**R 3**	**-1,4**	+0,6	+0,7	-0,3	**-1,0**
IgA Saliva	0,022	**-0,384**	-0,242	**-0,36**	-1,03	-0,68	-0,77	**-0,17**
Secretory IgA Saliva	-0,005	**-0,193**	0,150	**-1,23**	-1,38	-1,66	-1,34	**-1,24**
Immunity Integral Index	0,003	**-0,363**	-0,314	**+0,24**	-0,15	+0,10	-0,01	**+0,24**
IgA Serum	0,005	**-0,337**	-0,298	**+0,50**	-0,94	+0,13	-0,31	**+0,13**
Circulating Immune Complex	-0,001	**-0,309**	-0,017	**-0,17**	-0,62	-0,84	-0,60	**-0,11**
CD4⁺ T-helper Lymphocytes				**-0,89**	-1,87	-0,99	-1,39	**-0,55**
CD4⁺CD25⁺ T-regulatory Lym				**+0,95**	+1,85	+1,07	+1,74	**+0,47**
Monocytes	-0,013	**0,383**	-0,035	**-1,91**	+1,10	+0,78	-1,24	**-1,71**
Popovych's Strain Index-1	-0,002	**0,263**	0,197	**+0,18**	+1,43	+0,86	+1,18	**+0,26**
Erythrocyturia	0,005	**0,105**	0,060	**+1,12**	+1,46	+1,38	+1,42	**+1,25**
Lactobacilli feces				**-1,45**	-1,12	-0,97	-1,29	**-1,38**
Bifidobacteria feces				**-1,39**	-1,10	-1,02	-1,25	**-1,37**
Root 3 (2,2%)	**R 1**	**R 2**	**R 3**	-0,3	+0,3	**-1,2**	+1,0	-0,4
Phagocytose Index vs E. coli	0,021	-0,132	**-0,327**	+0,75	+0,27	**+1,11**	+0,50	+0,92
Killing Index vs Staph. aureus	0,026	0,192	**-0,279**	-1,53	-1,11	**-0,60**	-1,37	-1,01
Rod shaped Neutrophils	0,010	-0,103	**-0,249**	-2,24	-2,96	**-1,85**	-2,57	-2,32
Escherichia coli feces				-1,27	-0,93	**-0,85**	-1,02	-1,07
Leukocyturia	-0,043	-0,196	**0,108**	+1,49	+0,70	**+0,23**	+0,71	+0,56

The clear separation of the AH II cluster along the axis of the major root reflects the accompaniment of maximum BP levels by elevated levels of pro-inflammatory cytokines and normal, but minimal for the sample, activity and intensity of phagocytosis of gram-positive bacteria (Table 4 and Fig. 2).

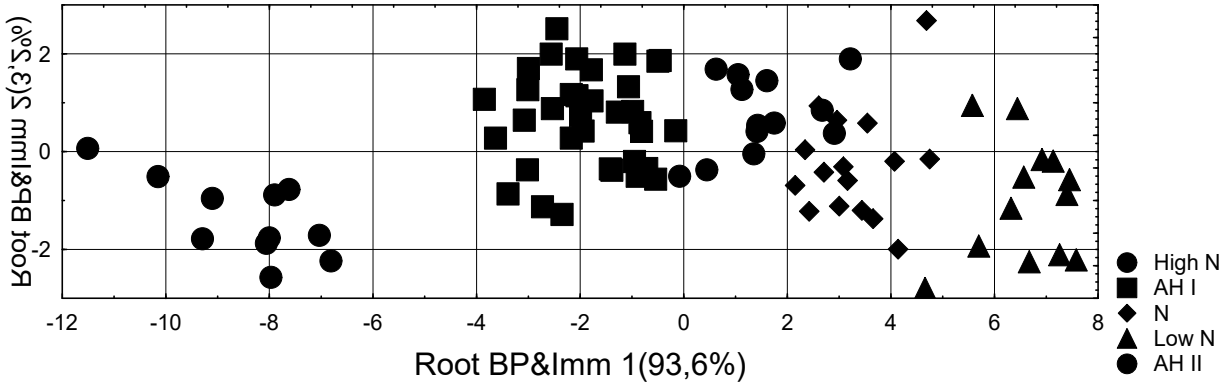

Fig. 2. Scattering of individual values of the first&second discriminant roots of patients of different blood pressure clusters

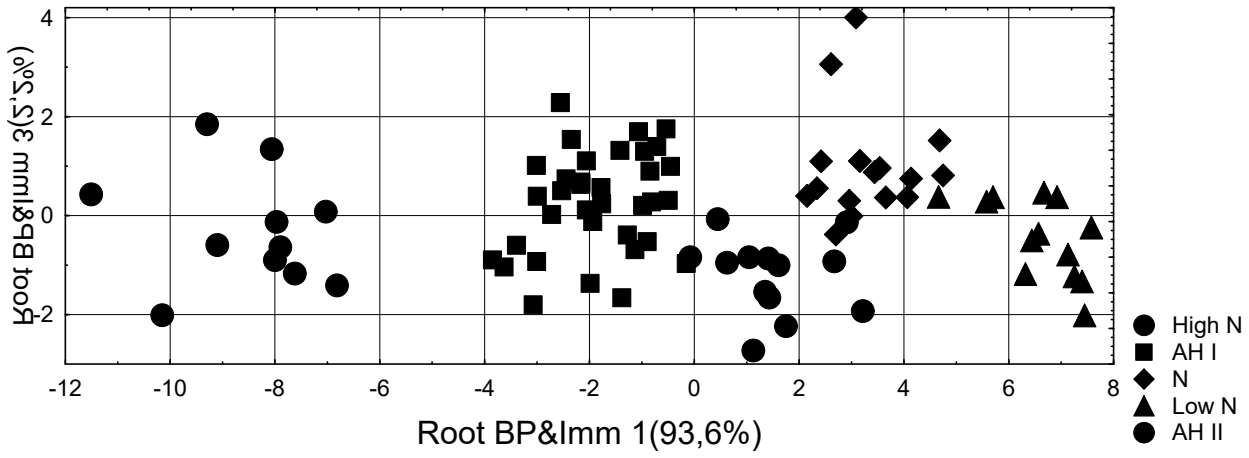

Fig. 3. Scattering of individual values of the first&third discriminant roots of patients of different blood pressure clusters

A cluster of patients with minimal BP is located at the opposite pole of the axis of the major root. However, the other variables mentioned are not min/max (extreme) for the sample. However, both extreme clusters are separated from the other three along the axis of the second root. Their lowest localization reflects their normal, but minimal for the sample, levels of serum and saliva IgA, circulating immune complexes, integral immunity index as well as maximally reduced levels of salivary secretory IgA and blood T-helper subpopulations - on the one hand, while minimally increased levels of regulatory T-lymphocytes and erythrocyturia, normal, but minimal for the sample leukocytary strain-index as well as maximally reduced levels of monocytes in the blood and probiotics in the intestines/feces - on the one hand.

Finally, patients with High Norm BP are distinguished from others along the axis of the third root. Their lowest localization reflects a minimally reduced blood level of rod shaped neutrophils and a minimally reduced bactericidal activity of neutrophils against Staphylococcus aureus as well as a maximally increased activity of phagocytosis by blood neutrophils of Escherichia coli and a minimally reduced content of the latter in the intestines/feces. This is accompanied by the minimum for the sample leukocyturia level.

In general, all clusters on the planes of three roots are clearly delineated, which is documented by calculating the Mahalanobis distances (Table 5).

Table 5. Squared Mahalanobis Distances between Blood Pressure Clusters and F-values (df=18,7; for High N-N $p<10^{-3}$; for Low N-N $p<10^{-5}$; for other pairs $p<10^{-6}$)

Blood Pressure Clusters	High Norm	AH I	Norm	Low Norm	AH II
High Norm	0	**14,2**	**9,07**	**30,8**	**105**
AH I	**5,94**	0	**28,6**	**74,5**	**48,8**
Norm	**2,87**	**13,9**	0	**14,7**	**142**
Low Norm	**8,83**	**31,2**	**4,31**	0	**228**
AH II	**27,7**	**18,0**	**40,4**	**40,9**	0

The same discriminant parameters can be used to identify the belonging of one or another person to one or another blood pressure cluster (Table 6).

Table 6. Coefficients and Constants for Classification Functions for Blood Pressure Clusters

Blood Pressure Clusters	High N	AH I	Norm	Low N	AH II
Variables currently in the model	p=,148	p=,398	p=,182	p=,148	p=,125
BP Systolic, mmHg	7,486	8,301	7,021	6,219	9,954
Immunity Integral Index	-101,9	-98,53	-97,99	-103,2	-91,06
Monocytes, %	13,16	12,87	12,85	13,15	12,18
Interleukin-6, ng/L	-1,335	-0,691	-1,136	-1,897	1,266
Leukocyturia, lgLeu/L	121,2	123,7	119,3	116,5	129,9
Circulating Immune Complex, units	-0,036	-0,009	-0,026	0,041	-0,004
Tumor Necrosis Factor-α, ng/L	7,977	6,871	7,072	8,483	4,775
BP Diastolic, mHg	0,840	0,713	0,860	0,969	0,378
Rod shaped Neutrophils, %	2,198	0,873	1,282	1,863	-0,975
Killing Index vs Staph. aureus, %	4,826	4,716	4,497	4,628	4,742
IgA Serum, g/L	-38,87	-43,05	-41,42	-37,45	-46,31
Phagocytose Index vs St. aureus, %	120,4	121,2	120,5	118,1	122,2
Secretory IgA Saliva, g/L	-0,633	-0,608	-0,595	-0,610	-0,608
IgA Saliva, g/L	-2,292	-2,321	-2,327	-2,255	-2,318
Popovych's Strain Index-1	-115,1	-118,4	-112,3	-115,3	-134,7
Microbian Count for St. aureus, B/Ph	-8,149	-8,302	-8,279	-8,056	-8,470
Phagocytose Index vs E. coli, %	83,59	82,41	82,73	84,03	81,00
Erythrocyturia, lgEr/L	-19,82	-22,98	-17,94	-14,42	-29,21
Constants	-10430	-10465	-10264	-10116	-10628

In this case, we can retrospectively recognize patients with high norm and low norm BP with two and one mistakes while others patients unmistakably. Overall classification accuracy is 96,6% (Table 7).

Table 7. Classification Matrix for Blood Pressure Clusters

	Classification Matrix (Struk_44.STA) Rows: Observed classifications Columns: Predicted classifications					
Group	Percent Correct	High N p=,14773	AH I p=,39773	N p=,18182	Low N p=,14773	AH II p=,12500
High N	84,6	11	1	1	0	0
AH I	100,0	0	35	0	0	0
N	100,0	0	0	16	0	0
Low N	92,3	0	0	1	12	0
AH II	100,0	0	0	0	0	11
Total	96,6	11	36	18	12	11

DISCUSSION

The results obtained in this study are consistent with existing data summarized in a number of excellent reviews [1,14,15,19-21]. Numerous cells of the immune system, both innate and adaptive immunity, have been indicated to play an important role in the development and maintenance of hypertension. In response to hypertensive stimuli such as Ang II and high salt, T cells become pro-inflammatory and they infiltrate the brain, blood vessel adventitia and periadventitial fat, heart, and the kidney. Pro-inflammatory T cell-derived cytokines such as IFN-γ and TNF-α (from $CD8^+$ and $CD4^+$Th1) and IL-17A (from the γδ-T cell and $CD4^+$Th17) exacerbate hypertensive responses mediating both endothelial

dysfunction. Th-1 and Th-17 effectors participate in inflammation which leads to increased blood pressure. One part of $CD4^+$ is the regulatory T cells (Tregs) that suppress immune response activation as they produce immunosuppressive cytokines, such as TGF-β and IL-10. Moreover, cross-talk among natural killer cells, adaptive immune cells (T cells and B cells), and innate immune cells (i.e. monocytes, macrophages, neutrophils, and dendritic cells) contributes to end-cardiovasculature damage and dysfunction in hypertension. Clinical and experimental studies on the diagnostic potential of T-cell subsets revealed that blood regulatory T cells, CD4 cells, and CD8 T cells show promise as biomarkers of hypertension. Therapeutic interventions to suppress activation of these cells may prove beneficial in reducing end-organ damage and preventing consequences of cardiovascular failure, including hypertension.

The above mostly applies to patients with AH II, while in persons with AH I and quasi-normal BP, its immune support is less pronounced.

CONCLUSION

Thus, a wide range of blood pressure in Truskavets' spa patients is accompanied by an equally wide range of metabolic, neural, endocrine and immune parameters. A detailed analysis and discussion will be conducted in next article.

ACKNOWLEDGMENT

We express sincere gratitude to colleagues of sanatoria "Kryshtalevyi Palats" and "Moldova" for help in conducting this investigation.

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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