“MYROSLAVA” AND “KHRYSTYNA” DRINKING MINERAL WATERS MODULATE THE STATE OF NEUROENDOCRINE-IMMUNE COMPLEX AND METABOLISM IN PATIENTS OF TRUSKAVETS’ SPA

Anatoliy I. Gozhenko¹, Myroslava V. Hrytsak¹,², Dariya V. Popovych³, Nataliya S. Badiuk¹,⁴, Xawery Żukow⁵

¹SE Ukrainian Research Institute of Medicine of Transport, Odesa, Ukraine
²Scientific group of Balneology of Hotel&Spa Complex "Karpaty", Truskavets’, Ukraine
³IY Horbachevs’kyi National Medical University, Ternopil’, Ukraine
⁴International Medical University, Odesa, Ukraine
⁵Medical University of Białystok, Białystok, Poland

Background. Earlier in an experiment on rats we showed that newly created sulfate-chloride sodium-magnesium drinking mineral waters "Myroslava" and "Khrystyna" of Truskavets’ spa has a significant modulating effects on the parameters of metabolism and the autonomic nervous, endocrine and immune systems. Adhering to the principle "Ex experimento ad clinic", we continued research in this direction with the participation of patients of the resort. We showed that these mineral waters have favorable effects on metabolic, HRVs, EEGs, endocrine and immune parameters. This article presents an integrated assessment of previously identified effects. Materials and Methods. The object of clinical-physiological observation were 34 men aged 23-70 years, who underwent rehabilitation treatment of chronic cholecystitis and pyelonephritis in remission in the Truskavets’ spa. The examination was performed twice, before and after a 7-10-day course of balneotherapy. All patients received bioactive water Naftussya, however, 11 men additionally drank water "Khrystyna", and the other 11 men water "Myroslava". The subject of the study were the parameters of the neuroendocrine-immune complex and metabolism. Results. The complex balneotherapy by interval use of sulfate-chloride sodium-magnesium mineral waters with Naftussya water causes significant changes in the constellation of EEGs, HRVs, endocrine, metabolic and immune parameters, which are different from the effects of Naftussya water monotherapy. Own effects of mineral waters are estimated by modeling. In general, the effects are physiologically favorable and have a normalizing nature. Conclusion. The newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets’ spa have favorable neuroendocrine, metabolic and immune effects on patients with chronic cholecystitis and pyelonephritis.

Keywords: sulfate-chloride sodium-magnesium drinking mineral waters, Truskavets’ spa, EEg, HRV, hormones, metabolism, immunity.

INTRODUCTION

Earlier in an experiment on rats, carried out in line with the concepts of neuroendocrine-immune complex [4,16,17,19,20] and functional-metabolic continuum [3], we showed that newly created sulfate-chloride sodium-magnesium drinking mineral waters "Myroslava" (5...
g/L) and "Khrystyna" (10 g/L) of Truskavets’ spa has a significant modulating effects on the parameters of metabolism and the autonomic nervous and endocrine systems [5-7, 18] as well as immunity [1]. Adhering to the principle "Ex experimento ad clinica", we continued research in this direction with the participation of patients of the resort. We showed that these mineral waters have favorable effects on metabolic, HRVs, EEGs, endocrine and immune parameters [8, 9]. This article presents an integrated assessment of previously identified effects.

**MATERIALS AND METHODS**

The object of clinical-physiological observation were 34 men aged 23-70 years, who underwent rehabilitation treatment in the Truskavets’ spa of chronic cholecystitis and pyelonephritis in remission with neuroendocrine-immune complex dysfunction. The examination was performed twice, before and after a 7-10-day course of balneotherapy. All patients received bioactive water Naftussya (3 ml/kg one hour before meals three times a day), however, 11 men in half an hour additionally drank water "Khrystyna", and the other 11 men - water "Myroslava" in the same dose.

The subject of the study were the EEGs [9], HRVs, endocrine, metabolic and immune [8] parameters.

**RESULTS AND DISCUSSION**

Following the accepted algorithm, the method of discriminant analysis [10] revealed 35 parameters, according to which the conditions of patients before and after the two balneotherapy regimens differ significantly. Characteristic were 14 EEGs, 2 HRVs, 2 endocrine, 4 immune and 13 metabolic parameters (Tables 1 and 2).

**Table 1. Summary of the analysis of discriminant functions in relation to the parameters of EEG**

| Variables currently in the model | Groups (n) and Means±SE | Parameters of Wilks' Statistics | Parameters of Wilks' Statistics |
|---------------------------------|-------------------------|---------------------------------|---------------------------------|
|                                | Before therapy (34)     | After Naftussya (12)            | After SW and N (22)             | Wilks’ Λ | Partial Λ | F-remove (2.31) | p-level | Tolerance | Norm Cv |
| Phosphates Excretion, mM/24 h   | 18,2 ± 1,2              | 16,8 ± 1,8                      | 42,4 ± 3,8                     | 0,013    | 0,538     | 13,3             | 10^-4   | 0,187     | 25,2     |
|                                | 25,2 ± 2,94             |                                 |                                |          |           |                  |         |           |          |
| Calcitonin, ng/L               | 6,95 ± 0,62             | 6,16 ± 1,11                     | 10,48 ± 1,21                   | 0,009    | 0,778     | 4,41             | 0,021   | 0,404     | 13,95    |
|                                | 13,95 ± 0,493           |                                 |                                |          |           |                  |         |           |          |
| Killing Index vs Staph. aur., %| 48,2 ± 1,5              | 45,2 ± 1,9                      | 57,7 ± 1,4                     | 0,011    | 0,631     | 9,04             | 0,001   | 0,286     | 58,9     |
|                                | 58,9 ± 0,142            |                                 |                                |          |           |                  |         |           |          |
| Testosterone, nM/L             | 18,5 ± 1,6              | 9,0 ± 1,0                       | 15,3 ± 2,1                     | 0,013    | 0,568     | 11,8             | 10^-4   | 0,286     | 14,8     |
|                                | 14,8 ± 0,400            |                                 |                                |          |           |                  |         |           |          |
| Magnesium Urine, mM/L          | 2,40 ± 0,11             | 2,14 ± 0,23                     | 2,22 ± 0,13                    | 0,011    | 0,655     | 8,16             | 0,001   | 0,055     | 2,93     |
|                                | 2,93 ± 0,256            |                                 |                                |          |           |                  |         |           |          |
| Creatinine Plasma, µM/L        | 92,6 ± 2,6              | 81,9 ± 2,8                      | 87,4 ± 2,0                     | 0,011    | 0,657     | 8,10             | 0,001   | 0,352     | 79,5     |
|                                | 79,5 ± 0,167            |                                 |                                |          |           |                  |         |           |          |
| Laterality β, %                | -3 ± 5                  | -33 ± 10                        | -4 ± 4                         | 0,008    | 0,925     | 1,25             | 0,301   | 0,286     | -6       |
|                                | -6 ± 28                 |                                 |                                |          |           |                  |         |           |          |
| Sodium Plasma, mM/L            | 141,5 ± 1,5             | 146,7 ± 2,1                     | 142,3 ± 2,0                    | 0,012    | 0,613     | 9,79             | 0,001   | 0,254     | 145,0    |
|                                | 145,0 ± 0,034           |                                 |                                |          |           |                  |         |           |          |
| LD Cholesterol Plasma, mM/L    | 3,54 ± 0,18             | 3,43 ± 0,032                    | 3,25 ± 0,21                    | 0,010    | 0,729     | 5,77             | 0,007   | 0,269     | 3,44     |
|                                | 3,44 ± 0,192            |                                 |                                |          |           |                  |         |           |          |
| Interleukin-6, ng/L            | 4,45 ± 0,36             | 3,67 ± 0,56                     | 4,58 ± 0,33                    | 0,011    | 0,673     | 7,52             | 0,002   | 0,146     | 4,25     |
|                                | 4,25 ± 0,324            |                                 |                                |          |           |                  |         |           |          |
|                | Entropy Fp2 | Sodium Urine, mM/L | Chloride Urine mM/L | Phosphate Plasma, mM/L | CD3⁺ active T-Lymphocytes, % | Lithogenicity Urine | T4-0 PSD, μV²/Hz | Laterality 0, % | C3-α PSD, % | P3-α PSD, % | Sodium Excretion, mM/24 h | Magnesium Excretion, mM/24 h | Chloride Excretion, mM/24 h | HF HRV PS, msec² | Glucose Plasma, mM/L | F8-6 PSD, % | Fp2-β PSD, μV²/Hz | T5-6 PSD, μV²/Hz | F4-β PSD, μV²/Hz | Entropy T6 | Microbiolar Count for St. aur., B/Ph | Laterality α, % | Entropy F7 |
|----------------|-------------|-------------------|---------------------|------------------------|-----------------------------|------------------------|------------------|----------------|-------------|-------------|---------------------------|--------------------------|-----------------------------|------------------|-----------------|-------------|-----------------|-----------------|-----------------|----------|-----------------|----------------|----------|
|                | 0.817       | 0.024             | 0.04                | 0.05                   | 0.08                        | 0.10                   | 0.12             | 0.14           | 0.16        | 0.18        | 0.21                      | 0.24                     | 0.27                        | 0.30             | 0.31            | 0.33        | 0.36            | 0.37            | 0.39            | 0.40     | 0.42            | 0.44          | 0.46     |
|                | 7.1         | 9.8               | 1.0                 | 8.8                    | 0.010                       | 0.748                   | 5.23              | 0.11           | 0.12        | 7.9         | 0.568                     | 969                      | 869                        | 141              | 628             | 740         | 869            | 119             | 127            | 14       | 85              | 85            | 85     |
|                | 0.07        | 1.0               | 1.3                 | 1.3                    | 0.010                       | 0.730                   | 5.72              | 0.008          | 0.259       | 1250        | 0.572                     | 0.010                    | 0.036                       | 0.048             | 0.008           | 0.04         | 0.036           | 0.04           | 0.045          | 0.04     | 0.05            | 0.05          | 0.05 |
|                | 0.790       | 0.834             | 0.026               | 0.710                   | 0.009                       | 0.801                   | 3.86              | 0.032          | 0.126       | 0.761        | 0.249                     | 62.8                     | 66.0                        | 2.0               | 60.2            | 2.3          | 2.3             | 2.0             | 2.0             | 2.0     | 2.0             | 2.0           | 2.0   |
|                | 0.031       | 0.851             | 0.024               | 0.724                   | 0.008                       | 0.911                   | 1.52              | 0.235          | 0.127       | 0.751        | 0.282                     | -1                       | -23                         | 9                | -18             | 5            | 5               | 9               | 9                | 9       | 9               | 9             | 9     |
|                | 0.039       | 0.851             | 0.024               | 0.724                   | 0.008                       | 0.911                   | 1.52              | 0.235          | 0.127       | 0.751        | 0.282                     |

Note. In each column, the first line is the average, the second – SE for variables and Cv or SD for Norm.
Table 2. Summary of step-by-step analysis of discriminant variables ranked by criterion Λ

| Variables currently in the model | F to enter | p-level | Λ F-value | p-level |
|---------------------------------|------------|---------|-----------|---------|
| Phosphates Excretion, mM/24 h   | 33.0       | 10^-5   | 0.496     | 33.0    | 10^-6 |
| Calcitonin, ng/L                | 7.42       | 0.001   | 0.403     | 18.4    | 10^-6 |
| Killing Index vs Staph. aureus, % | 6.14      | 0.004   | 0.337     | 15.2    | 10^-6 |
| Testosterone, nM/L              | 5.86       | 0.005   | 0.283     | 13.6    | 10^-6 |
| Magnesium Urine, mM/L           | 5.57       | 0.006   | 0.240     | 12.7    | 10^-6 |
| Creatinine Plasma, µM/L         | 6.31       | 0.003   | 0.198     | 12.5    | 10^-6 |
| Laterality β, %                 | 6.32       | 0.003   | 0.163     | 12.4    | 10^-6 |
| Sodium Plasma, mM/L             | 4.61       | 0.014   | 0.141     | 12.1    | 10^-6 |
| LD Cholesterol Plasma, mM/L     | 3.61       | 0.033   | 0.125     | 11.6    | 10^-6 |
| Interleukin-6, ng/L             | 4.05       | 0.023   | 0.109     | 11.4    | 10^-6 |
| F7-0 PSD, %                     | 4.09       | 0.022   | 0.095     | 11.2    | 10^-6 |
| VLF HRV PS, msec²               | 2.99       | 0.058   | 0.085     | 10.9    | 10^-6 |
| Entropy Fp2                     | 3.84       | 0.028   | 0.075     | 10.8    | 10^-6 |
| Sodium Urine, mM/L              | 3.43       | 0.040   | 0.066     | 10.7    | 10^-6 |
| Chloride Urine mM/L             | 2.49       | 0.094   | 0.060     | 10.5    | 10^-6 |
| Phosphate Plasma, mM/L          | 2.81       | 0.069   | 0.054     | 10.3    | 10^-6 |
| CD3⁺ active T-Lymphocytes, %    | 1.76       | 0.183   | 0.050     | 9.96    | 10^-6 |
| Lithogenicity Urine             | 2.51       | 0.092   | 0.046     | 9.82    | 10^-6 |
| T4-0 PSD, µV²/Hz                | 2.33       | 0.109   | 0.042     | 9.66    | 10^-6 |
| Laterality 0, %                 | 4.06       | 0.024   | 0.035     | 9.94    | 10^-6 |
| C3-α PSD, %                     | 2.03       | 0.144   | 0.032     | 9.76    | 10^-6 |
| P3-α PSD, %                     | 2.36       | 0.106   | 0.029     | 9.70    | 10^-6 |
| Sodium Excretion, mM/24 h       | 1.58       | 0.218   | 0.027     | 9.46    | 10^-6 |
| Magnesium Excretion, mM/24 h    | 4.18       | 0.022   | 0.023     | 9.86    | 10^-6 |
| Chloride Excretion, mM/24 h     | 2.95       | 0.063   | 0.020     | 10.0    | 10^-6 |
| HF HRV PS, msec²                | 2.62       | 0.085   | 0.018     | 10.1    | 10^-6 |
| Glucose Plasma, mM/L            | 2.97       | 0.063   | 0.015     | 10.3    | 10^-6 |
| F8-δ PSD, %                     | 1.73       | 0.191   | 0.014     | 10.1    | 10^-6 |
| Fp2-β PSD, µV²/Hz               | 1.62       | 0.211   | 0.013     | 9.98    | 10^-6 |
| T5-δ PSD, µV²/Hz                | 1.48       | 0.241   | 0.012     | 9.82    | 10^-6 |
| F4-β PSD, µV²/Hz                | 1.78       | 0.184   | 0.011     | 9.75    | 10^-6 |
| Entropy T6                      | 1.73       | 0.192   | 0.010     | 9.69    | 10^-6 |
| Microbian Count for St. aureus, B/Ph | 1.60 | 0.218   | 0.009     | 9.59    | 10^-6 |
| Laterality α, %                 | 2.02       | 0.149   | 0.008     | 9.64    | 10^-6 |
| Entropy F7                      | 1.52       | 0.235   | 0.007     | 9.55    | 10^-6 |

A number of variables despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 3).
Table 3. Variables not included in the model

| Variables                        | Groups (n) and Means±SE | Parameters of Wilks' Statistics |
|----------------------------------|--------------------------|---------------------------------|
|                                  | Before therapy (34)      |                                 |
|                                  | After Naftus-sya (12)    |                                 |
|                                  | After Salt Waters and N (22) |                                |
| Deviation δ, Hz                 | 0.73 ± 0.05              | 0.71 ± 0.10                     |
|                                  |                          | 0.57 ± 0.04                     |
|                                  |                          | 0.007                           |
| Fp2-0 PSD, μV²/Hz, %             | 9.7 ± 1.5                | 8.9 ± 0.8                       |
|                                  |                          | 6.7 ± 1.3                       |
|                                  |                          | 0.007                           |
| F4-α PSD, %, μV²/Hz              | 29 ± 7                   | 18 ± 3                          |
|                                  |                          | 20 ± 4                          |
| F7-δ PSD, μV²/Hz                 | 31.4 ± 3.4               | 22.0 ± 3.8                      |
|                                  |                          | 31.5 ± 3.1                      |
| F8-α PSD, μV²/Hz                 | 37 ± 4                   | 37 ± 13                         |
| T4-β PSD, %                     | 29.0 ± 2.4               | 33.6 ± 4.7                      |
|                                  |                          | 37.3 ± 4.6                      |
| T6-δ PSD, %, μV²/Hz              | 174 ± 73                 | 53 ± 11                         |
| Entropy P3                       | 0.819 ± 0.022            | 0.843 ± 0.029                   |
|                                  |                          | 0.736 ± 0.030                   |
| P3-δ PSD, %, %                  | 27.3 ± 3.3               | 27.5 ± 4.9                      |
| Entropy O2                       | 0.769 ± 0.028            | 0.798 ± 0.027                   |
|                                  |                          | 0.669 ± 0.037                   |
| O2-δ PSD, μV²/Hz                 | 272 ± 117                | 104 ± 19                        |
| Potassium Urine, mM/L           | 39.5 ± 3.2               | 41.5 ± 3.6                      |
|                                  |                          | 30.5 ± 1.7                      |
| (Ca/K)<sup>+</sup> as S/V balance | 0.728 ± 0.012           | 0.729 ± 0.014                   |
|                                  |                          | 0.708 ± 0.010                   |
| Interleukin-1, ng/L              | 4.94 ± 0.19              | 4.36 ± 0.37                     |
|                                  |                          | 5.17 ± 0.30                     |
| Aldosterone, pM/L                | 225 ± 5                  | 236 ± 0.37                      |
| ULF HRV PS, msec²               | 73 ± 15                  | 139 ± 56                        |

Norm Cv/σ: 0.66, 0.405, 0.66, 0.405
The identifying information contained in the 35 discriminant variables is condensed into two roots. The major root contains 71% of discriminatory opportunities ($r^* = 0.971$; Wilks' $\Lambda = 0.0072$; $\chi^2(70) = 237; p<10^{-6}$), while minor root 29% only ($r^* = 0.934$; Wilks' $\Lambda = 0.127$; $\chi^2(34) = 99; p<10^{-6}$).

Calculating the values of discriminant roots for each patient by coefficients and constants given in Table 4 allows visualization of each patient in the information space of roots (Fig. 1).

### Table 4. Standardized and raw coefficients and constants for discriminant variables

| Variables                        | Coefficients | Standardized | Raw |
|----------------------------------|--------------|--------------|-----|
|                                  | Root 1       | Root 2       | Root 1 | Root 2 |
| Phosphates Excretion, mM/24 h    | 1.447        | -0.749       | 0.124 | -0.064 |
| Calcitonin, ng/L                 | 0.760        | -0.067       | 0.172 | -0.015 |
| Killing Index vs Staph. aureus, %| 1.123        | -0.334       | 0.148 | -0.044 |
| Testosterone, nM/L               | -0.771       | -1.043       | -0.089 | -0.120 |
| Magnesium Urine, mM/L            | 1.093        | -2.423       | 1.649 | -3.655 |
| Creatinine Plasma, µM/L          | -0.989       | -0.241       | -0.078 | -0.019 |
| Laterality $\beta$, %            | -0.305       | -0.445       | -0.012 | -0.018 |
| Sodium Plasma, mM/L              | 0.135        | 1.313        | 0.015 | 0.149 |
| LD Cholesterol Plasma, mM/L      | -0.894       | 0.543        | -0.857 | 0.521 |
| Interleukin-6, ng/L              | -0.368       | -1.556       | -0.192 | -0.813 |
| F7-0 PSD, %                      | 0.512        | 1.407        | 0.124 | 0.342 |
| VLF HRV PS, msec$^2$             | 0.936        | 0.496        | 0.0015 | 0.0008 |
| Entropy Fp2                      | -0.111       | -0.520       | -0.762 | -3.557 |
| Sodium Urine, mM/L               | -3.648       | -1.215       | -0.120 | -0.040 |
| Chloride Urine mM/L              | 0.173        | 3.030        | 0.005 | 0.085 |
| Phosphate Plasma, mM/L           | 0.052        | 0.099        | 0.279 | 0.527 |
| CD3$^+$ active T-Lymphocytes, %   | -0.603       | 0.072        | -0.130 | 0.016 |
| Lithogenicity Urine              | 0.673        | -0.558       | 4.746 | -3.934 |
| T4-0 PSD, µV$^2$/Hz              | -0.476       | -0.863       | -0.017 | -0.032 |
| Laterality $\theta$, %           | -0.389       | 1.942        | -0.011 | 0.054 |
| C3-α PSD, %                      | -2.167       | -0.498       | -0.139 | -0.032 |
| P3-α PSD, %                      | 1.787        | -0.101       | 0.101 | -0.006 |
| Sodium Excretion, mM/24 h         | 3.994        | 1.081        | 0.043 | 0.012 |
| Magnesium Excretion, mM/24 h      | -3.408       | 2.714        | -1.950 | 1.553 |
| Chloride Excretion, mM/24 h       | 0.528        | -2.613       | 0.0056 | -0.0278 |
| HF HRV PS, msec$^2$               | -0.758       | -0.079       | -0.0014 | -0.0001 |
| Glucose Plasma, mM/L             | 0.514        | 0.136        | 0.537 | 0.142 |
| F8-δ PSD, %                      | -0.192       | 0.087        | -0.007 | 0.003 |
| Fp2-β PSD, µV$^2$/Hz              | -0.996       | -0.870       | -0.026 | -0.022 |
| T5-δ PSD, µV$^2$/Hz               | -0.532       | -0.275       | -0.0007 | -0.0003 |
| F4-β PSD, µV$^2$/Hz               | 0.857        | 0.181        | 0.0188 | 0.0040 |
| Entropy T6                       | -0.298       | -1.309       | -1.901 | -8.342 |
| Microbian Count for St. aureus, Bac/Phag | -0.126 | 0.592 | -0.015 | 0.071 |
| Laterality $\alpha$, %           | 0.159        | -1.125       | 0.006 | -0.042 |
| Entropy F7                       | -0.085       | 0.892        | -0.450 | 4.748 |

| Constants                        | 4.70         | -8.79        |
| Cumulative Proportion            | 0.708        | 1            |

Following the accepted algorithm, Table 5 collects the Z-scores of discriminant variables together with those that are not included in the model, but still reflect the specifics of the water used.
Pseudo-staining visualizes a combination of neuroendocrine, metabolic and immune parameters in the structure of each root (Table 5), consistent with previously identified neuroendocrine-immune and neuroendocrine-metabolic linkages [11-17,19,21-23].

Table 5. Correlations between variables and roots, centroids of clusters and Z-scores of clusters

| Variables | Correlations Variables-Roots | Before therapy (44) | After Naftus-sya (12) | After Salt Waters and N (22) |
|-----------|-----------------------------|---------------------|----------------------|-----------------------------|
| Root 1 (70.8%) | Root 1 Root 2 | -3.27 -1.11 | +5.66 |
| Calcitonin | 0.094 -0.055 | -1.02 -1.14 | -0.51 |
| Triiodothyronine | 0.050 -0.039 | -0.85 -0.55 | -0.46 |
| VLF HRV PS | 0.035 -0.048 | +0.07 -0.15 | +0.45 |
| P3-α PSDr | 0.038 0.002 | -0.04 -0.03 | +0.82 |
| Cholecystokinetic Activity | | | |
| Killing Index vs Staph. aureus | 0.148 -0.104 | -1.28 -1.64 | -0.15 |
| Phosphates Excretion | 0.240 -0.093 | -0.94 -1.14 | +2.31 |
| Magnesium Excretion | 0.111 -0.115 | +0.29 -0.64 | +1.79 |
| Chloride Excretion | 0.087 -0.011 | +0.62 +1.02 | +3.16 |
| (UA•Ca)/(Cr•Mg)²₅ Lithogenicity Urine | 0.072 -0.058 | +0.59 +0.43 | +0.98 |
| Potassium Plasma | -0.72 -0.64 | -0.25 |
| Calcium Urine | -1.18 -1.09 | -0.13 |
| Phosphates Urine | -1.41 -1.36 | -0.42 |
| Laterality θ | -0.063 -0.052 | -0.04 -0.66 | -0.98 |
| Entropy Fp2 | -0.060 0.004 | +0.13 -0.02 | -0.66 |
| T4-0 PSDa | -0.042 -0.043 | +0.03 -0.12 | -0.16 |
| (Ca/K)²⁵ Plasma as Symp/Vagal balance | | | |
| Phosphate Plasma | -0.081 0.096 | -0.82 -0.36 | -1.43 |
| Potassium Urine | -0.56 -0.40 | -1.27 |
| Sodium Urine | -0.111 0.010 | +0.39 +0.17 | -0.90 |
| Uric Acid Urine | +0.30 -0.24 | -0.65 |
| LD Cholesterol Plasma | -0.031 -0.006 | +0.16 -0.11 | -0.28 |
| Glucose Plasma | -0.021 -0.007 | +0.09 -0.02 | -0.15 |
| Root 2 (29.2%) | Root 1 Root 2 | -1.47 +5.49 | -0.72 |
| Laterality β | 0.007 -0.167 | +0.10 -0.95 | +0.08 |
| Laterality α | -0.045 -0.096 | +0.12 -0.71 | -0.52 |
| Fp2-β PSDa | 0.004 -0.085 | +0.35 -0.26 | +0.35 |
| F4-β PSDa | 0.013 -0.060 | +0.30 -0.11 | +0.43 |
| C3-α PSDr | 0.020 -0.055 | +0.01 -0.29 | +0.20 |
| T5-δ PSDa | -0.013 -0.049 | +0.34 -0.14 | +0.09 |
| Testosterone | -0.029 -0.148 | +0.84 -0.82 | +0.24 |
| Parathyroid activity | | | |
| Interleukin-6 | 0.013 -0.062 | +0.14 -0.42 | +0.24 |
| Interleukin-1 | | +0.50 -0.24 | +0.78 |
| VLD Cholesterol Plasma | | +0.32 -0.21 | +0.09 |
| Creatinine Plasma | -0.037 -0.111 | +0.99 +0.18 | +0.60 |
| Urea Plasma | +0.83 +0.45 | +0.63 |
| Sodium Excretion | 0.022 -0.076 | +2.17 +0.78 | +2.58 |
| Magnesium Urine | -0.027 -0.047 | -0.71 -1.05 | -0.95 |
| Entropy F7 | 0.003 0.106 | -0.22 +0.47 | -0.13 |
| F7-0 PSDr | 0.029 0.078 | -0.11 +0.51 | +0.29 |
| Parameter                                      | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 |
|-----------------------------------------------|---------|---------|---------|---------|---------|
| F8-δ PSDr                                     | -0.034  | 0.072   | +0.02   | +0.45   | -0.37   |
| Entropy T6                                     | -0.042  | 0.055   | +0.15   | +0.39   | -0.27   |
| ULF HRV PS                                    | -0.45   | +0.16   | -0.11   |         |         |
| Aldosterone                                   | -0.30   | -0.05   | -0.19   |         |         |
| CD3 active T-Lymphocytes                       | -0.063  | 0.110   | -0.33   | +0.25   | -0.78   |
| Microbial Count for Staph. aureus             | -0.040  | 0.068   | +0.12   | +0.44   | -0.14   |
| Chloride Urine                                | -0.029  | 0.111   | -0.85   | +0.38   | -1.15   |
| Chloride Plasma                                | -0.26   | +1.00   | -0.07   |         |         |
| Sodium Plasma                                  | 0.003   | 0.083   | -0.71   | +0.33   | -0.55   |
| Calcium Plasma                                 | -0.66   | -0.44   | -0.64   |         |         |
| Creatinine Urine                              | -1.69   | -1.11   | -2.02   |         |         |

The localization in the extreme right zone of the axis of the first root of the cluster of patients who received two mineral waters shows a significant increase of initially reduced or minimum for sampling parameters that are positively associated with the root, and reduction of initially normal or even deeper fall of initially reduced parameters correlating with the root negatively. Instead, in patients receiving Naftussya water only, these parameters remained unchanged or changed to a much lesser extent.

On the other hand, such patients are characterized by a significant decrease/increase in another number of parameters associated with the second root negatively/positively, while in combination balneotherapy their changes are insignificant or much less pronounced.

Fig. 1. Scattering of individual values of the first and second discriminant roots of patients before (circles) and after the course of drinking only water Naftussya (triangles) and in combination with water "Myroslava" or "Khrystyna" (rhombuses)

Fig. 2 illustrates that the integrated initial state of all three groups of patients was almost the same as the effect on the discriminant variables of both sulfate-chloride sodium-magnesium mineral waters.
Fig. 2. Mean values (M±SD) of the first and second discriminant roots of patients before (red fill) and after the course of drinking only water "Naftussya" (circle) and in combination with water "Myroslava" (triangle) or "Khrystyna" (square).

The visual impression of a clear demarcation of the three clusters in the information field of the two roots is documented by calculating the distances of Mahalanobis (Table 6).

Table 6. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=35.3) and p-levels (below the diagonal)

| Clusters | Before therapy | After Naftussya | After SW&N |
|----------|----------------|----------------|------------|
| Before therapy | 0 | 53 | 80 |
| After Naftussya | 6.4 x 10^5 | 0 | 84 |
| After SW&N | 14.6 x 10^4 | 8.9 x 10^4 | 0 |

Selected discriminant variables were used to identify the affiliation of a patient to a particular cluster. This goal of discriminant analysis is realized with the help of classification functions (Table 7).

Table 7. Coefficients and constants of classification functions

| Variables | Clusters | Before therapy | After Naftussya | After Salt W&N |
|-----------|----------|----------------|----------------|---------------|
| Phosphates Excretion, mM/24 h | -1,132 | -0,310 | 0,929 |
| Calcitonin, ng/L | -0,193 | 0,073 | 1,332 |
| Killing Index vs Staph. aureus, % | 1,681 | 1,695 | 2,969 |
| Testosterone, nM/L | -1,552 | -2,580 | -2,434 |
| Magnesium Urine, mM/L | -17,24 | -39,09 | -5,234 |
| Creatinine Plasma, µM/L | 0,956 | 0,654 | 0,243 |
| Laterality β, % | 0,346 | 0,195 | 0,223 |
| Sodium Plasma, mM/L | 6,044 | 7,115 | 6,292 |
The calculation of algebraic differences between the mean Z-scores of the parameters in both groups of patients still allows us to assess the partial effects of sulfate-chloride sodium-magnesium mineral waters.

This approach suggests that sulfate-chloride sodium-magnesium mineral waters have their own (per se) more or less pronounced effect on the constellation of parameters of the neuro-endocrine-immune complex and metabolism, regardless of their initial levels (Fig. 3).

| Parameter                              | Baseline | Salt Water |
|----------------------------------------|----------|------------|
| LD Cholesterol Plasma, mM/L            | 27.72    | 29.49      | 20.46      |
| Interleukin-6, ng/L                    | -15.60   | -21.67     | -17.92     |
| F7-0 PSD, %                           | -8.705   | -6.057     | -7.339     |
| VLF HRV PS, msec                       | 0.036    | 0.045      | 0.050      |
| Entropy Fp2                            | 161.3    | 134.9      | 151.9      |
| Sodium Urine, mM/L                     | 0.716    | 0.181      | -0.382     |
| Chloride Urine mM/L                    | 1.289    | 1.891      | 1.395      |
| Phosphate Plasma, mM/L                 | 159.5    | 163.7      | 162.3      |
| CD3\(^+\) active T-Lymphocytes, %      | 4.920    | 4.748      | 3.772      |
| Lithogenicity Urine                    | 135.3    | 118.2      | 174.7      |
| T4-0 PSD, μV²/Hz                       | 0.758    | 0.501      | 0.579      |
| Laterality 0, %                        | -0.953   | -0.599     | -1.010     |
| C3-α PSD, %                           | 2.162    | 1.639      | 0.896      |
| P3-α PSD, %                           | -2.219   | -2.041     | -1.324     |
| Sodium Excretion, mM/24 h              | -0.041   | 0.132      | 0.349      |
| Magnesium Excretion, mM/24 h           | 26.65    | 33.23      | 10.39      |
| Chloride Excretion, mM/24 h            | -0.398   | -0.579     | -0.368     |
| HF HRV PS, msec²                       | -0.049   | -0.052     | -0.061     |
| Glucose Plasma, mM/L                   | 22.68    | 24.82      | 27.57      |
| F8-δ PSD, %                           | 0.186    | 0.193      | 0.124      |
| Fp2-β PSD, μV²/Hz                      | 0.506    | 0.295      | 0.260      |
| T5-δ PSD, μV²/Hz                       | 0.041    | 0.037      | 0.034      |
| F4-β PSD, μV²/Hz                       | -0.399   | -0.331     | -0.228     |
| Entropy T6                             | 1.772    | -60.33     | -21.40     |
| Microbian Count for St. aureus, Bac/Phag | 0.501  | 0.962     | 0.420     |
| Laterality α, %                        | 1.040    | 0.759      | 1.062      |
| Entropy F7                             | 233.8    | 265.8      | 233.3      |

Costants: -1041, -1102, -1015

**Fig. 3.** Profiles of real Z-scores of initial EEG, HRV, endocrine, immune and metabolic variables and their simulated Z-scores after consumption of sulphate-chloride sodium-magnesium mineral waters
In particular, initially reduced neuroendocrine (VLF band HRV, calcitonin, triiodothyronine) and metabolic (urine concentrations of phosphate, calcium, magnesium and creatinine, phosphaturia, plasma potassium and calcium, cholecystokinin activity) variables as well as the completion of phagocytosis of Staphylococcus aureus increase, as a rule, to the zone of norm. On the other hand, initially increased urinary excretion and concentration of sodium and plasma creatinine and urea levels are reduced. Such effects are consistent with the ancient concept of the ambivalent-balancing nature of the effects of balneal factors on the body [2].

However, there are an increase in initially normal levels of PSD of alpha rhythm in loci P3 and C3, laterality and PSD of beta rhythm in loci Fp2 and F4, vagal tone, plasma testosterone, parathyroid activity, excretion of magnesium and chloride, plasma interleukins 6 and 1, as well as a decrease in initially normal levels of EEG entropy in loci Fp2 and T6, Ca/K marker of sympathetic-vagal balance, concentration of uric acid in urine as well as LD cholesterol in plasma as well as the intensity of Staphylococcus aureus phagocytosis. The latter pattern is formed by initially reduced EEG entropy in locus F7, PSD of delta rhythm in locus F8, plasma levels of phosphate and chloride, chloride and potassium of urine, as well as active T-lymphocytes of blood, which continue to decline. Such effects do not fit into this concept, but are consistent with the known data on the diversity of responses of the neuroendocrine-immune complex and metabolism to balneal factors [15,23].

**CONCLUSION**

The newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets resort have favorable neuroendocrine, metabolic and immune effects on patients with chronic cholecystitis and pyelonephritis.

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**ACCORDANCE TO ETHICS STANDARDS**

Tests in patients are carried out 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.

**REFERENCES**

1. Badiuk NS, Popovych DV, Hrytsak MV, Ruzhylo SV, Zakalyak NR, Kovalchuk GY, Mel’nyk OI, Zukow X. Similar and specific immunotrophic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets’ spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(11): 314-333.

2. Balanov's'kyi VP, Popovych IL, Karpynets’ SV. About ambivalence-equilibratory character of influence of curative water Naftussya on organism of human [in Ukrainian]. Dopovidi ANU. Mat pryr tekhn Nauky. 1993; 3: 154-158.

3. Gozhenko AI. Functional-metabolic continuum [in Russian]. J of NAMS of Ukraine. 2016; 22 (1): 3-8.

4. Gozhenko AI, Korda MM, Popadynets’ OO, Popovych IL. Entropy, Harmony, Synchronization and Their Neuro-Endocrine-Immune Correlates [in Ukrainian]. Odesa. Feniks; 2021:
5. Hrytsak MV, Barylyak LG, Usyn's'kyi RS, Mysula IR. Endocrine and metabolic effects of sulfate chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets' spa in healthy female rats. In: Proceedings of the XII All-Ukrainian scientific-practical conference "Topical issues of pathology under the influence of extraordinary factors on the body". Galician Readings II (Ternopil', October 29-30, 2020). Ternopil'; 2020: 125-127.

6. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow W. Similar neuroendocrine and metabolic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets’ spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(6): 320-334.

7. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow W. Peculiaritias of neuroendocrine and metabolic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets’ spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(9): 862-875.

8. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow X. Comparative study of the effect on the neuroendocrine-immune complex and metabolism of drinking monotherapy with Naftussya water and therapy supplemented with “Myroslava” and “Khrystyna” mineral waters. Journal of Education, Health and Sport. 2022; 12(1): 355-367.

9. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow X. Comparative study of the effects on the EEG of drinking monotherapy with Naftussya water and therapy supplemented with “Myroslava” and “Khrystyna” mineral waters. Journal of Education, Health and Sport. 2022; 12(2): 141-150.

10. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika; 1989: 78-138.

11. Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 3. Correlations between parameters EEG, HRV and Immunogram. Journal of Education, Health and Sport. 2017; 7(3): 53-71.

12. Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Kyjenko VM, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 2. Correlations between parameters EEG, HRV and Phagocytosis. Journal of Education, Health and Sport. 2017; 7(9): 439-459.

13. Kul'chyns'kyi AB, Kyjenko VM, Zukow W, Popovych IL. Causal neuro-immune relationships at patients with chronic pyelonephritis and cholecystitis. Correlations between parameters EEG, HRV and white blood cell count. Open Medicine. 2017; 12(1): 201-213.

14. Kul'chyns'kyi AB, Zukow W, Korolyshyn TA, Popovych IL. Interrelations between changes in parameters of HRV, EEG and humoral immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017; 7(9): 439-459.

15. Kul'chyns'kyi AB, Zukow W. Three variants of immune responses to balneotherapy at the spa Truskavets’ in patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018; 8(3): 476-489.

16. Mel'nyk OI, Zukow W, Hrytsak MV, Popovych DV, Zavidnyuk YV, Bilas VR, Popovych IL. Canonical analysis of neuroendocrine-metabolic and neuroendocrine-immune relationships at female rats. Journal of Education, Health and Sport. 2021; 11(5): 356-369.

17. Polovynenko IS, Zayats LM, Zukow W, Popovych IL. Neuro-endocrine-immune relationships by chronic stress at male rats. Journal of Health Sciences. 2013; 3(12): 365-374.

18. Popovych IL. Functional interactions between neuroendocrine-immune complex in males rats [in Ukrainian]. Achievements of Clinical and Experimental Medicine. 2008; 2(9): 80-87.

19. Popovych IL. The concept of neuroendocrine-immune complex (Review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18

20. Popovych DV, Badiuk NS, Hrytsak MV, Ruzhylo SV, Mel’nyk OI, Zukow X. Sulfate-chloride sodium-magnesium mineral waters modulate neuroendocrine-immune complex and metabolism in healthy female rats. Journal of Education, Health and Sport. 2021; 11(12): 455-466.

21. Popovych IL, Kul’chyns’kyi AB, Korolyshyn TA, Zukow W. Interrelations between changes in parameters of HRV, EEG and cellular immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017; 7(10): 11-23.
22. Popovych IL, Kul’chyns’kyi AB, Gozhenko AI, Zukow W, Kovbasnyuk MM, Korolyshyn TA. Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018; 8(2): 135-156.

23. Vis’tak HI, Popovych IL. Vegetotropic effects of bioactive water Naftussya and their endocrine and immune support in female rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 39-57.