Comparative study of the effect on the neuroendocrine-immune complex and metabolism of drinking monotherapy with Naftussya water and therapy supplemented with “Myrosława” and “Khrystyna” mineral waters

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Background. Earlier in an experiment on rats, we showed that the newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets’ spa have neuroendocrine and metabolic effects significantly different from daily water. Adhering to the principle "From experiment to clinic", we continued research in this direction with the participation of patients of the resort. 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 resort. 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 "Myrosława". 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 Naftusya water causes significant changes in the constellation of neuroendocrine, metabolic and immune parameters, which are different from the effects of Naftusya 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 resort have favorable neuroendocrine, metabolic and immune effects on patients with chronic cholecystitis and pyelonephritis.
INRODUCTION

Earlier in an experiment on rats, we showed that the newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets’ spa have neuroendocrine and metabolic effects significantly different from daily water [11-13]. Adhering to the principle "Ex experimento ad clinic", we continued research in this direction with the participation of patients of the resort.

MATERIALS AND METHODS

The object of clinical-physiological observation were 34 men aged 23-70 years, who underwent rehabilitation treatment in the Truskavets resort of chronic cholecystitis and pyelonephritis in remission with of 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 day before, daily urine was collected, in which was determined the concentration of electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (flaming photometry); nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method). Urine lithogenicity index (Lith) was also calculated by the Tiselius’ HS [24] formula modified by Flyunt VR et al [7]:

\[
\text{Lith} = (\text{Uric acid} \cdot \text{Calcium/Magnesium} \cdot \text{Creatinine})^{0.25}.
\]

The same metabolic parameters were determined in plasma as well as glucose (glucose-oxidase method), triglycerides (by a certain meta-periodate method), total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of \( \alpha \)-lipoproteins (by the enzyme method after precipitation of noto-lipoproteins); prae-\( \beta \)-lipoproteins (expected by the level of triglycerides); \( \beta \)-lipoproteins (expected by a difference between a total cholesterol and cholesterol in composition \( \alpha \)-and prae-\( \beta \)-lipoproteins).

The analysis carried out according to instructions [8] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents.

According to the parameters of Ca and phosphates exchange, parathyroid activity was evaluated by coefficient \((\text{Cap}\cdot \text{Pu}/\text{Cau}\cdot \text{Pr})^{0.22}\), based on its classical effects and recommendations by Popovych IL [9] as well as evaluated sympato-vagal balance by coefficient \((\text{Cap}/\text{Kp})^{0.5} [6]\).

We determined content in plasma major hormones of adaptation: Cortisol, Testosterone and Triiodothyronine (by the ELISA with the use of analyzer “RT-2100C” and corresponding sets of reagents from “Алкор Біо”, XEMA Co., Ltd and DRG International Inc.).

In basal conditions we estimated the state of the autonomous regulation by the method heart rate variability (HRV) [1,3,10,23], using a hardware-programmatic complex "CardioLab+HRV" (KhAI Medica, Kharkiv, Ukraine). The following parameters were subject to analysis. Frequency Domain Methods: HF (0,4÷0,15 Hz), LF (0,15÷0,04 Hz), VLF
(0.04÷0.015 Hz), ULF (0.015÷0.003 Hz) компоненти. Time Domain Methods: HR, SDNN, RMSSD, pNN\textsubscript{50}. Calculated as well as Kerdö’s Vegetative Index [5] and the entropy (h) of the relative spectral powers (SP) of the HRV bands by the formula Popovych IL [9]:

$$h_{HRV} = \frac{\text{SPHF} \cdot \log_2 \text{SPHF} + \text{SPLF} \cdot \log_2 \text{SPLF} + \text{SPVLF} \cdot \log_2 \text{SPVLF} + \text{SPULF} \cdot \log_2 \text{SPULF}}{\log_2 4}$$

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manual [19]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, 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 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”), Interleukins 1\textbeta and 6 (ELISA, analyser “Stat Fax 303”, USA, reagents from “Vector-Best”, RF).

In portion of capillary the blood we counted up Leukocytogram and calculated the Entropy (h) of Leukocytogram (LCG) as well as Immunoctogram (ICG) using IL Popovych’s formulas [9]:

$$h_{LCG} = - [L \cdot \log_2 L + M \cdot \log_2 M + E \cdot \log_2 E + \text{SNN} \cdot \log_2 \text{SNN} + \text{StubN} \cdot \log_2 \text{StubN}] \log_2 5$$

$$h_{ICG} = - [\text{CD4} \cdot \log_2 \text{CD4} + \text{CD8} \cdot \log_2 \text{CD8} + \text{CD22} \cdot \log_2 \text{CD22} + \text{CD56} \cdot \log_2 \text{CD56}] \log_2 4$$

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [4] with moderately modification by MM Kovbasnyuk [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. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC “Truskavets’kurort”. 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 recorded partial parameters of Phagocytosis, taking into account the Neutrophils (N) content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula [9]:

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

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”) applied [20,21]. To quantify cholekinetics, the area between the cholecystovolumogram and the basal line was calculated.

Normal (reference) values of variables are taken from the database of the Truskavetsian School of Balneology.

For statistical analysis used the software package "Statistica 64".

**RESULTS AND DISCUSSION**

Following the accepted algorithm, in this study, two research groups were combined to determine the effects common to both sulfate-magnesium mineral waters.

In order to identify those indicators for which the condition of patients on admission to treatment and after monotherapy or complex balneotherapy differ significantly, a discriminant analysis [16] of registered indicators was conducted. The program included in the discriminant model 27 variables, including 15 metabolic, 7 neuroendocrine and 5 immune (Tables 1 and 3).
Table 1. Summary of the analysis of discriminant functions in relation to the parameters of metabolism and neuro-endocrine-immune complex

| Variables currently in the model | Before therapy (34) | After Naftusya (12) | After Salt Waters and N (22) | Wilks’ Λ | Partial Λ | F-remove (2,4) | p-level | Tolerance | Norm Cv (30) |
|---------------------------------|---------------------|---------------------|-----------------------------|---------|-----------|----------------|---------|-----------|-------------|
| Phosphates Excretion, mM/24 h   | 18,2 1,2            | 16,8 1,8            | 42,4 3,8                   | 0,050   | 0,428     | 25,3 10⁻⁴     | 0,233   | 25,2      | 0,294       |
| Calcitonin, ng/L                | 6,95 0,62           | 6,16 1,11           | 10,48 1,21                 | 0,029   | 0,747     | 6,42 0,004    | 0,427   | 13,95     | 0,493       |
| Creatinine Plasma, μM/L         | 92,6 2,6            | 81,9 2,8            | 87,4 2,0                   | 0,033   | 0,654     | 10,1 10⁻³     | 0,426   | 79,5      | 0,167       |
| Testosterone, nM/L              | 18,5 1,6            | 9,0 1,0             | 15,3 2,1                   | 0,036   | 0,602     | 12,6 10⁻⁴     | 0,359   | 14,8      | 0,400       |
| Sodium Plasma, mM/L             | 141,5 1,5           | 146,7 2,1           | 142,3 2,0                  | 0,031   | 0,699     | 8,19 0,001    | 0,260   | 145,0     | 0,034       |
| Phosphate Plasma, mM/L          | 1,04 0,03           | 1,13 0,06           | 0,91 0,04                  | 0,028   | 0,785     | 5,20 0,010    | 0,274   | 1,20      | 0,167       |
| Magnesium Ure, mM/L             | 2,40 0,11           | 2,14 0,23           | 2,22 0,13                  | 0,027   | 0,816     | 4,28 0,021    | 0,095   | 2,93      | 0,256       |
| Chloride Excretion, mM/24 h     | 186 13              | 197 15              | 259 27                     | 0,023   | 0,936     | 1,30 0,0284   | 0,025   | 167,5     | 0,172       |
| Interleukin-6, ng/L             | 4,45 0,36           | 3,67 0,56           | 4,58 0,33                  | 0,026   | 0,843     | 3,54 0,039    | 0,240   | 4,25      | 0,324       |
| LD Cholesterol Plasma, mM/L     | 3,54 0,36           | 3,43 0,32           | 3,25 0,21                  | 0,032   | 0,670     | 9,35 10⁻³     | 0,237   | 3,44      | 0,192       |
| Sodium Ure, mM/L                | 119 5               | 114 8               | 89 7                       | 0,031   | 0,689     | 8,57 0,001    | 0,010   | 110       | 0,211       |
| Microbian Count for St. aur., B/Ph | 62,8 1,2           | 66,0 2,0            | 60,2 2,3                   | 0,024   | 0,883     | 2,52 0,094    | 0,624   | 61,6      | 0,160       |
| Glucose Plasma, mM/L            | 4,77 0,17           | 4,68 0,33           | 4,59 0,18                  | 0,027   | 0,807     | 4,55 0,017    | 0,532   | 4,70      | 0,160       |
| Chloride Ure, mM/L              | 102 3               | 127 14              | 96 10                      | 0,026   | 0,840     | 3,63 0,036    | 0,027   | 120       | 0,172       |
| Sodium Excretion, mM/24 h        | 225 18              | 179 11              | 238 19                     | 0,029   | 0,743     | 6,56 0,004    | 0,014   | 154       | 0,211       |
| [(Ca/K)]⁸⁵ as Sympt-Vagal balance | 0,728 0,012       | 0,729 0,014         | 0,708 0,010                | 0,023   | 0,928     | 1,48 0,240    | 0,194   | 0,710     | 0,104       |
| VLF HRV PS, msec⁷               | 969 99              | 869 141             | 1238 168                   | 0,025   | 0,860     | 3,09 0,057    | 0,319   | 1250      | 0,572       |
| HF HRV PS, msec⁷                | 354 75              | 407 262             | 541 100                    | 0,024   | 0,900     | 2,12 0,134    | 0,206   | 350       | 0,713       |
| Magnesium Excretion, mM/24 h    | 4,40 0,29           | 3,43 0,36           | 5,98 0,43                  | 0,031   | 0,703     | 8,04 0,001    | 0,035   | 4,10      | 0,256       |
| Lithogenicity Ure              | 0,86 0,03           | 0,83 0,05           | 0,95 0,03                  | 0,028   | 0,770     | 5,67 0,007    | 0,443   | 0,73      | 0,300       |
| Killing Index vs Staph. aur., % | 48,2 1,5            | 45,2 1,9            | 57,7 1,4                   | 0,026   | 0,833     | 3,81 0,031    | 0,375   | 58,9      | 0,142       |
| CD³⁺ active T-Lymphocytes,%    | 28,3 0,8            | 31,3 0,9            | 26,1 1,1                   | 0,026   | 0,817     | 4,27 0,021    | 0,470   | 30,0      | 0,167       |
| Interleukin-1, ng/L            | 4,94 0,19           | 4,36 0,37           | 5,17 0,30                  | 0,022   | 0,964     | 0,72 0,495    | 0,613   | 4,51      | 0,173       |
| Potassium Ure, mM/L            | 39,5 3,2            | 41,5 3,6            | 30,5 1,7                   | 0,026   | 0,827     | 3,99 0,027    | 0,022   | 46,4      | 0,269       |
| Aldosterone, pM/L              | 225 5               | 236 10              | 229 4                      | 0,025   | 0,861     | 3,06 0,058    | 0,023   | 258       | 0,187       |
| ULF HRV PS, msec⁷              | 73 15               | 139 56              | 110 34                     | 0,024   | 0,908     | 1,92 0,161    | 0,331   | 122       | 0,892       |
| HD Cholesterol Plasma, mM/L    | 1,35 0,08           | 1,41 0,14           | 1,31 0,08                  | 0,023   | 0,949     | 1,02 0,370    | 0,458   | 1,34      | 0,300       |

Note. In each column, the first line is the average, the second – SE or Cv.
A number of variables, primarily **cholecystokinin activity** to the standard stimulus, despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 2).

**Table 4.2. Metabolic and neuroendocrine-immune complex parameters not included in the model**

| Variables                          | Before therapy (34) | After Naftusyxa (12) | After Salt Waters and N (22) | Wilks’s Lambda | Partial Lambda | F to enter | p-level | Tolerance | Norm CV (30) |
|------------------------------------|---------------------|----------------------|-----------------------------|----------------|----------------|------------|---------|-----------|--------------|
| Cholecystokinin Activity, units    | 553 (34)            | 584 (12)             | 675 (22)                    | 0.021          | 0.982          | 0.34       | 0.715   | 0.572     | 624 (0.131)  |
| Calcium Urine, mM/L                | 2.34 ± 0.18         | 2.40 ± 0.82          | 3.04 ± 0.26                 | 0.022          | 0.995          | 0.10       | 0.910   | 0.354     | 3.13 ± 0.214 |
| Phosphates Urine, mM/L             | 10.8 ± 0.7          | 10.5 ± 1.1           | 15.8 ± 1.3                  | 0.021          | 0.975          | 0.47       | 0.629   | 0.071     | 18.0 ± 0.294 |
| Potassium Plasma, mM/L             | 4.21 ± 0.10         | 4.25 ± 0.16          | 4.43 ± 0.10                 | 0.021          | 0.990          | 0.19       | 0.831   | 0.457     | 4.55 ± 0.104 |
| Uric Acid Urine, mM/L              | 2.33 ± 0.23         | 1.93 ± 0.10          | 1.79 ± 0.12                 | 0.021          | 0.979          | 0.39       | 0.681   | 0.344     | 2.14 ± 0.250 |
| VLF HRV PS, %                      | 50.8 ± 3.0          | 51.1 ± 6.5           | 44.4 ± 2.5                  | 0.021          | 0.980          | 0.37       | 0.691   | 0.346     | 53.9 ± 0.277 |
| Triiodothyronine, nM/L             | 1.97 ± 0.13         | 1.93 ± 0.30          | 1.78 ± 0.13                 | 0.022          | 0.996          | 0.07       | 0.932   | 0.142     | 2.20 ± 0.227 |
| Chloride Plasma, mM/L              | 100.8 ± 1.0         | 105.3 ± 1.2          | 101.3 ± 1.6                 | 0.022          | 0.999          | 0.02       | 0.980   | 0.044     | 101.5 ± 0.032 |
| CD4+ T-helper Lymphocytes, %        | 28.0 ± 1.3          | 34.8 ± 2.1           | 26.7 ± 0.9                  | 0.022          | 0.999          | 0.02       | 0.980   | 0.044     | 39.5 ± 0.082 |
| CD8+ T- cytolytic Lymphocytes, %    | 22.6 ± 0.8          | 24.3 ± 1.5           | 21.5 ± 1.0                  | 0.021          | 0.974          | 0.49       | 0.613   | 0.057     | 23.5 ± 0.138 |
| VLD Cholesterol Plasma, mM/L       | 0.57 ± 0.05         | 0.48 ± 0.08          | 0.64 ± 0.08                 | 0.022          | 0.999          | 0.02       | 0.980   | 0.474     | 0.54 ± 0.612 |
| LF HRV PS, msec²                   | 717 ± 101           | 691 ± 213            | 1604 ± 158                  | 0.021          | 0.965          | 0.06       | 0.519   | 0.189     | 625 ± 0.482  |
| Calcium Plasma, mM/L               | 2.20 ± 0.04         | 2.23 ± 0.04          | 2.20 ± 0.04                 | 0.022          | 0.997          | 0.06       | 0.943   | 0.169     | 2.30 ± 0.065 |
| Urea Plasma, mM/L                  | 5.60 ± 0.17         | 6.17 ± 0.19          | 6.04 ± 0.26                 | 0.021          | 0.984          | 0.31       | 0.737   | 0.442     | 5.00 ± 0.330 |
| Creatinine Urine, mM/L             | 3.9 ± 0.3           | 5.2 ± 0.6            | 3.1 ± 0.3                   | 0.021          | 0.969          | 0.60       | 0.554   | 0.246     | 7.9 ± 0.300  |
| Cortisol, nM/L                     | 373 ± 26            | 441 ± 30             | 419 ± 41                    | 0.021          | 0.993          | 0.13       | 0.875   | 0.716     | 405 ± 0.524  |
| Parathyroid activity, units        | 1.81 ± 0.06         | 1.73 ± 0.07          | 1.90 ± 0.04                 | 0.022          | 0.999          | 0.37       | 0.519   | 0.189     | 1.82 ± 0.230 |
| Blood Pressure systolic, mmHg      | 141.2 ± 2.7         | 141.9 ± 5.3          | 141.4 ± 3.5                 | 0.021          | 0.978          | 0.42       | 0.663   | 0.493     | 124.5 ± 0.076 |
| Blood Pressure diastolic, mmHg     | 84.6 ± 1.7          | 85.7 ± 1.7           | 86.0 ± 8.2                  | 0.021          | 0.987          | 0.25       | 0.780   | 0.476     | 79.0 ± 0.054 |
| Kerdoe Vegetative Index, units     | -18.8 ± 3.4         | -23.1 ± 5.1          | -19.5 ± 3.8                 | 0.021          | 0.969          | 0.13       | 0.663   | 0.442     | -23.5 ± 20.1 |
Table 3. 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    | 10⁻⁵    |
| Calcitonin, ng/L                 | 7,42      | 0,001   | 0,403 | 18,4    | 10⁻⁶    |
| Creatinine Plasma, µM/L          | 5,95      | 0,004   | 0,279 | 13,8    | 10⁻⁶    |
| Testosterone, nM/L               | 5,86      | 0,005   | 0,234 | 13,0    | 10⁻⁶    |
| Sodium Plasma, mM/L              | 5,68      | 0,005   | 0,197 | 12,5    | 10⁻⁶    |
| Phosphate Plasma, mM/L           | 5,55      | 0,006   | 0,166 | 12,3    | 10⁻⁶    |
| Magnesium Urine, mM/L            | 5,20      | 0,008   | 0,140 | 12,1    | 10⁻⁶    |
| Chloride Excretion, mM/24 h      | 4,11      | 0,022   | 0,123 | 11,7    | 10⁻⁶    |
| Interleukin-6, ng/L              | 3,38      | 0,041   | 0,110 | 11,3    | 10⁻⁶    |
| LD Cholesterol Plasma, mM/L      | 3,83      | 0,028   | 0,096 | 11,1    | 10⁻⁶    |
| Sodium Urine, mM/L               | 2,83      | 0,068   | 0,087 | 10,8    | 10⁻⁶    |
| Microbian Count for Staph. aur., Bac/Ph | 2,49    | 0,092   | 0,080 | 10,4    | 10⁻⁶    |
| Glucose Plasma, mM/L             | 2,44      | 0,097   | 0,073 | 10,1    | 10⁻⁶    |
| Chloride Urine mM/L              | 2,51      | 0,092   | 0,066 | 9,81    | 10⁻⁶    |
| Sodium Excretion, mM/24 h        | 2,05      | 0,140   | 0,061 | 9,51    | 10⁻⁶    |
| (Ca/K)²⁵ Plasma as Symp/Vagal balance | 1,75    | 0,185   | 0,057 | 9,18    | 10⁻⁶    |
| VLF HRV PS, msec²                | 1,40      | 0,255   | 0,054 | 8,81    | 10⁻⁶    |
| HF HRV PS, msec²                 | 4,41      | 0,018   | 0,045 | 9,13    | 10⁻⁶    |
| Magnesium Excretion, mM/24 h     | 2,16      | 0,127   | 0,042 | 8,99    | 10⁻⁶    |
| (UA•Ca)/(Cr•Mg)²⁵/Lithogenicity Urine | 3,04    | 0,058   | 0,037 | 9,06    | 10⁻⁶    |
| Killing Index vs Staph. aur., %   | 2,17      | 0,126   | 0,033 | 8,96    | 10⁻⁶    |
| CD³⁺ active T-Lymphocytes, %     | 2,33      | 0,109   | 0,030 | 8,92    | 10⁻⁶    |
| Interleukin-1, ng/L              | 1,10      | 0,341   | 0,029 | 8,61    | 10⁻⁶    |
| Potassium Urine, mM/L            | 1,17      | 0,321   | 0,027 | 8,34    | 10⁻⁶    |
| Aldosterone, pM/L                | 1,54      | 0,226   | 0,025 | 8,18    | 10⁻⁶    |
| ULF HRV PS, msec²                | 1,95      | 0,155   | 0,023 | 8,12    | 10⁻⁶    |
| HD Cholesterol Plasma, mM/L      | 1,02      | 0,370   | 0,022 | 7,87    | 10⁻⁶    |

The identifying information contained in the 27 discriminant variables is condensed into two roots. The major root contains 80% of discriminatory opportunities (r*=0,958; Wilks’ Λ=0,022; χ²(56)=197; p<10⁻⁶), while minor root - 20% only (r*=0,857; Wilks’ Λ=0,265; χ²(27)=68; p<10⁻⁴).

Calculating the values of discriminant roots for each patient as the sum of the products of non-standardized (raw) coefficients for individual values of discriminant variables together with the constant (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                   | -1.619       | 0.244        | -0.1389 | 0.0210 |
| Calcitonin, ng/L                               | -0.802       | 0.038        | -0.1817 | 0.0085 |
| Creatinine Plasma, µM/L                        | 0.857        | -0.435       | 0.0678  | -0.0344 |
| Testosterone, nM/L                             | 0.389        | -1.150       | 0.0448  | -0.1325 |
| Sodium Plasma, mM/L                            | -0.434       | 1.159        | -0.0493 | 0.1318 |
| Phosphate Plasma, mM/L                         | -0.0586      | 1.031        | -0.3130 | 5.5092 |
| Magnesium Urine, mM/L                          | -1.217       | -0.886       | -1.8362 | -1.3358 |
| Chloride Excretion, mM/24 h                    | 0.666        | -1.712       | 0.0071  | -0.0182 |
| Interleukin-6, ng/L                            | -0.161       | -0.926       | -0.0841 | -0.4839 |
| LD Cholesterol Plasma, mM/L                    | 1.059        | 0.701        | 1.0159  | 0.6727 |
| Sodium Urine, mM/L                             | 5.833        | -0.777       | 0.1913  | -0.0255 |
| Microbian Count for Staph. aur., Bac/Ph        | 0.4101       | 0.2130       | 0.0491  | 0.0255 |
| Glucose Plasma, mM/L                           | -0.502       | 0.423        | -0.5240 | 0.4415 |
| Chloride Urine mM/L                            | -1.351       | 2.433        | -0.0379 | 0.0683 |
| Sodium Excretion, mM/24 h                      | -4.335       | 1.370        | -0.0463 | 0.0146 |
| (Ca/K)² Plasma as Symp/Vagal balance           | -0.566       | 0.327        | -9.7651 | 5.6385 |
| VLF HRV PS, msec²                              | -0.440       | 0.596        | -0.0007 | 0.0009 |
| HF HRV PS, msec²                               | 0.596        | -0.468       | 0.0011  | -0.0008 |
| Magnesium Excretion, mM/24 h                   | 2.973        | 0.751        | 1.7012  | 0.4299 |
| (UA•Ca)/(Cr•Mg)² Lithogenicity Urine           | -0.714       | -0.262       | -5.0313 | -1.8465 |
| Killing Index vs Staph. aur., %                | -0.624       | -0.346       | -0.0821 | -0.0455 |
| CD3+ active T-Lymphocytes, %                    | 0.325        | 0.632        | 0.0700  | 0.1363 |
| Interleukin-1, ng/L                            | -0.191       | 0.187        | -0.1547 | 0.1310 |
| Potassium Urine, mM/L                          | -2.663       | -1.391       | -0.1784 | -0.0932 |
| Aldosterone, pM/L                              | 2.345        | 1.221        | 0.0876  | 0.0456 |
| ULF HRV PS, msec¹                               | -0.3119      | -0.5054      | -0.0023 | -0.0037 |
| HD Cholesterol Plasma, mM/L                    | 0.344        | 0.057        | 0.7764  | 0.1275 |
| **Constants**                                  | **-10.23**   | **-37.42**   | **0.802** | **1** |
| **Eigenvalues**                                | **11.26**    | **2.77**     |         |        |
| **Cumulative Proportion**                      | **0.802**    |              |         |        |

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

| Variables | Correlations Variables-Roots | After Salt Waters and N (22) | After Naftussya (12) | Before therapy (44) |
|-----------|-----------------------------|----------------------------|---------------------|---------------------|
| Root 1(80 %) | Root 1 Root 2  | -4.73 +1.87 +2.40 |
| Phosphates Excretion | -0.299 -0.063 +2.31 -1.14 -0.94 |
| Magnesium Excretion | -0.146 -0.141 +1.79 -0.64 +0.29 |
| Chloride Excretion | -0.106 0.012 +3.16 +1.02 +0.62 |
| (UA×Ca)/(Cr×Mg) Lithogenicity Urine | -0.094 -0.066 +0.98 +0.43 +0.59 |
| HF HRV PS | -0.045 0.016 +0.82 -0.03 -0.04 |
| Cholecystokinetic Activity | +0.62 -0.30 -0.86 |
| Killing Index vs Staph. aureus | -0.190 -0.111 -0.15 -1.64 -1.28 |
| Potassium Plasma | -0.25 -0.64 -0.72 |
| Calcium Urine | -0.13 -1.09 -1.18 |
| Phosphates Urine | -0.42 -1.36 -1.41 |
| Triiodothyronine | -0.46 -0.55 -0.85 |
| VLF HRV PS | -0.065 -0.043 -0.01 -0.47 -0.36 |
| Calcitonin | -0.119 -0.054 -0.51 -1.14 -1.02 |
| Phosphate Plasma | 0.108 0.122 -1.43 -0.36 -0.82 |
| Sodium Urine | 0.135 -0.022 -0.90 +0.17 +0.39 |
| Uric Acid Urine | -0.65 -0.24 +0.30 |
| Potassium Urine | 0.089 0.041 -1.27 -0.40 -0.56 |
| LD Cholesterol Plasma | 0.036 -0.019 -0.28 -0.11 +0.16 |
| Glucose Plasma | 0.025 -0.018 -0.15 -0.02 +0.09 |
| (Ca/K) Plasma as Symp/Vagal balance | 0.051 0.009 -0.05 +0.23 +0.24 |
| Root 2(20 %) | Root 1 Root 2 | -0.14 +3.39 -1.11 |
| Testosterone | 0.019 -0.241 +0.24 -0.82 +0.84 |
| Magnesium Urine | 0.027 -0.082 -0.95 -1.05 -0.71 |
| Interleukin-6 | -0.022 -0.093 +0.24 -0.42 +0.14 |
| Interleukin-1 | -0.040 -0.110 +0.78 -0.24 +0.50 |
| VLD Cholesterol Plasma | +0.09 -0.21 +0.32 |
| Parathyroid activity | +0.19 -0.22 -0.03 |
| Creatinine Plasma | 0.032 -0.185 +0.60 +0.18 +0.99 |
| Urea Plasma | +0.63 +0.45 +0.83 |
| Sodium Excretion | -0.035 -0.111 +2.58 +0.78 +2.17 |
| Chloride Urine | 0.047 0.164 -1.15 +0.38 -0.85 |
| Creatinine Urine | -2.02 -1.11 -1.69 |
| Chloride Plasma | -0.07 +1.00 -0.26 |
| CD3 active T-Lymphocytes | 0.088 0.151 -0.78 +0.25 -0.33 |
| Sodium Plasma | 0.005 0.130 -0.55 +0.33 -0.71 |
| Calcium Plasma | -0.64 -0.44 -0.66 |
| Microbion Count for Staph. aureus | 0.056 0.092 -0.14 +0.44 +0.12 |
| HD Cholesterol Plasma | 0.017 0.031 -0.08 +0.21 +0.04 |
| ULF HRV PS | -0.023 0.103 -0.11 +0.16 -0.45 |
| Aldosterone | -0.012 0.091 -0.19 -0.05 -0.30 |

The localization in the extreme left zone of the axis of the first root of the cluster of patients who received two mineral waters shows a significant increase relative to baseline levels of parameters that are negatively associated with the root, and a significant decrease in positively correlated with the root parameters. In contrast, 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 (squares) 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=15.4) and p-levels (below the diagonal)

| Clusters | Before therapy | After Naftussya | After SW&N |
|----------|----------------|----------------|------------|
| Before therapy | 0 | 21 | 52 |
| After Naftussya | $3.8 \times 10^4$ | 0 | 56 |
| After SW&N | $14.5 \times 10^9$ | $9.1 \times 10^4$ | 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

| Clusters | Before therapy | After Naftussya | After SW&N |
|----------|----------------|----------------|------------|
| Variables | p=.500 | p=.176 | p=.324 |
| Phosphates Excretion, mM/24 h | -0.742 | -0.574 | 0.270 |
| Calcitonin, ng/L | -2.613 | -2.478 | -1.308 |
| Creatinine Plasma, µM/L | -0.164 | -0.355 | -0.681 |
| Testosterone, nM/L | -2.692 | -3.312 | -3.141 |
| Sodium Plasma, mM/L | 5.563 | 6.182 | 6.043 |
| Phosphate Plasma, mM/L | 471.4 | 496.4 | 479.0 |
| Magnesium Ureine, mM/L | -41.67 | -46.71 | -29.86 |
| Chloride Excretion, mM/24 h | -0.108 | -0.194 | -0.176 |
| Interleukin-6, ng/L | -30.56 | -32.69 | -30.43 |
| LD Cholesterol Plasma, mM/L | 70.60 | 73.08 | 64.00 |
| Sodium Ureine, mM/L | 7.063 | 6.846 | 5.673 |
| Microbian Count for Staph. aur., Bac/Ph | 3.221 | 3.310 | 2.895 |
| Glucose Plasma, mM/L | 12.50 | 14.76 | 16.66 |
| Chloride Ureine mM/L | 0.682 | 1.009 | 1.019 |
| Sodium Excretion, mM/24 h | -0.511 | -0.420 | -0.166 |
| (Ca/K)²⁵ Plasma as Symp/Vagal balance | 149.1 | 179.7 | 224.2 |
| VLIF HRV PS, msec² | 0.091 | 0.095 | 0.097 |
| HF HRV PS, msec² | -0.088 | -0.092 | -0.097 |
| Magnesium Excretion, mM/24 h | 34.30 | 35.33 | 22.57 |
| (UA•Ca)/(Cr•Mg)²⁵ Lithogenicity Ureine | 40.54 | 34.90 | 74.66 |
| Killing Index vs Staph. aureus, % | -0.166 | -0.328 | 0.376 |
| CD3⁺ active T-Lymphocytes, % | 10.05 | 10.62 | 9.678 |
| Interleukin-1, ng/L | 7.842 | 8.604 | 9.93 |
| Potassium Ureine, mM/L | -19.99 | -20.32 | -18.81 |
| Aldosterone, pM/L | 10.02 | 10.18 | 9.436 |
| ULF HRV PS, msec² | -0.512 | -0.528 | -0.500 |
| HD Cholesterol Plasma, mM/L | 26.96 | 27.12 | 21.55 |

Constants | -2151 | -2319 | -2122 |
The use of classification functions allows unmistakable retrospective identification of all clusters (Table 8).

Table 8. Classification matrix
Rows: observed classifications; columns: projected classifications

|          | Percent Correct | Before therapy p=.500 | After Naftussya p=.176 | After Salt W&N p=.324 |
|----------|-----------------|------------------------|-------------------------|------------------------|
| Groups   |                 | Before therapy         | After Naftussya          | After Salt W&N          |
| Before   | 100             | 34                     | 0                       | 0                      |
| therapy  |                 |                         |                         |                        |
| After    | 100             | 0                      | 12                      | 0                      |
| Naftussya|                 |                         |                         |                        |
| After    | 100             | 0                      | 0                       | 22                     |
| Salt W&N |                 |                         |                         |                        |
| Total    | 100             | 34                     | 12                      | 22                     |

Thus, we have shown that complex balneotherapy by interval use of sulfate-chloride sodium-magnesium mineral water with Naftussya water causes significant changes in the constellation of neuroendocrine, metabolic and immune parameters, which are different from the effects of Naftussya water monotherapy.

In the conditions of the resort, it was organizationally (but also ethically) impossible to offer patients to use only newly created mineral waters. However, the calculation of algebraic differences between the mean Z-scores of the parameters in both groups of patients still allows us to assess the independent 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 neuroendocrine-immune complex and metabolism, regardless of their initial levels (Fig. 3).

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**Fig. 3. Profiles of real Z-scores of initial discriminant variables and their simulated Z-scores after consumption of sulphate-chloride sodium-magnesium mineral waters**

In particular, initially reduced neuroendocrine (VLF and ULF bands HRV, calcitonin, triiodothyronine, to a lesser extent aldosterone and parathyroid activity) and metabolic (urine
concentrations of phosphate, calcium, magnesium and creatinine, phosphaturia, plasma potassium and calcium, cholecystokinetic 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 is an increase in initially normal levels of vagal tone, parathyroid activity, excretion of magnesium and chloride and interleukins 1 and 6 plasma, as well as a decrease in initially normal levels of Ca/K marker sympathetic-vagal balance, concentration of uric acid in urine as well as glucose and cholesterol in plasma as well as the intensity of Staphylococcus aureus phagocytosis. The latter pattern is formed by initially reduced plasma levels of sodium, 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 [14,15,17,22,25].

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.

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