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RELATIONSHIPS BETWEEN CHANGES IN URIC ACID PARAMETERS METABOLISM AND PARAMETERS OF IMMUNITY AND MICROBIOTA IN PATIENTS WITH NEUROENDOCRINE-IMMUNE COMPLEX DYSFUNCTION

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Abstract

Background. Previously by screening correlation links between uricemia and uricosuria, on the one hand, and immunity and microbiota parameters, on the other, registered in patients with chronic pyelonephritis and cholecystitis in remission **twice** (before and after ten days of balneotherapy), we found that raw uricemia determines by 28% nine parameters of immunity as well as bacteriuria and content in E. coli feces. Uricemia, normalized by sex and age, determines by 25% another constellation of immunity parameters as well as content in E. coli feces with impaired enzymatic activity and Klebsiella&Proteus. Instead, uricosuria determines only four

parameters of immunity and only by 11,5%. The **purpose** of this study is to analyze the correlation between individual **changes** in the two parameter sets caused by balneotherapy. **Material and Methods.** The object of observation were 34 men and 10 women aged 24-70 years old, who came to the Truskavets' spa for the rehabilitation treatment of chronic pyelonephritis combined with cholecystitis in remission. The serum and daily urine levels of the uric acid by uricase method were determined. Immune status evaluated on a set of I and II levels recommended by the WHO. The condition of microbiota is evaluated on the results of sowing of feces and urine. **Results.** It is found that individual changes in uricemia correlate inversely with changes in the level of T-helpers in blood as well as IgA and IgS in saliva while positively with changes in the level of Monocytes with the almost same force as their static levels. Instead, inverse links with changes in inflammatory markers and positive connections with changes in CIC and T-killers that are absent with respect to static parameters are revealed. However, no association was found between changes in uricemia and the intensity of Staph. aureus phagocytosis by blood neutrophils. Changes in uricosuria correlate with changes in only three immune parameters, and even at the limit of significance. Taking into account other relationships, we find that balneotherapy-induced changes in uricemia and uricosuria determine changes in immune status by 60%. **Conclusion.** Endogenous uric acid has a modulating effects on a number of immune parameters in patients with neuroendocrine-immune complex dysfunction on background of chronic inflammatory diseases.

Key words: Uricemia; Uricosuria; Immunity; Microbiota; Relationships; Balneotherapy; Humans.

INTRODUCTION

Previously, continuing experimental studies [6-8,10], by screening correlation links between uricemia and uricosuria, on the one hand, and immunity and microbiota parameters, on the other, registered in each of 44 patients with chronic pyelonephritis and cholecystitis in remission **twice** (before and after ten days of balneotherapy), we found that raw uricemia determines by 28% nine parameters of immunity as well as bacteriuria and content in E. coli feces. Uricemia, normalized by sex and age, determines by 25% another constellation of immunity parameters as well as content in E. coli feces with impaired enzymatic activity and Klebsiela&Proteus. Instead, uricosuria determines only four parameters of immunity and only by 11,5% [9].

It is known that balneological agents of the Truskavets' spa affect both the immune system and the metabolism of uric acid [11,23,26], but the links between these effects have not been thoroughly analyzed.

The **purpose** of this study is to analyze the correlation between individual **changes** in the two parameter sets caused by balneotherapy.

MATERIAL AND METHODS

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

remission. The survey was conducted **twice**, before and after ten-day balneotherapy (drinking Naftussya bioactive water three times a day, ozokerite applications, mineral baths every other day) [23,26].

We adduce data by OR Dats'ko et al [3] about organic compounds (in mg/L) water Naftussya. Paraffins 4,10÷4,20; monoolefins 1,67÷1,75; dienes and monocycloolefins 0,84÷0,85; alkylbenzene 1,55÷1,54; alkenylbenzene 0,47÷0,46; esters of aromatic acids 1,32÷1,33; alkyl phenols 1,14÷1,14; polyaromatic hydrocarbons 0,077÷0,059; oxygene-containing connections (acids) 1,12÷1,14; sulfur-containing connections 0,30÷0,31; alkyl naphthalenes 0,53÷0,53; unidentified polyaromatic hydrocarbons 0,19÷0,19; connections required subsequent identification 0,48÷0,50 correspondingly. Early have been shown that detected in Naftussya phenols (0,5÷4,1 µg/L) comed from fallen leaves [14].

The serum and daily urine levels of the uric acid by uricase method were determined. The analyzes were carried out according to the instructions described in the manual [5]. The analyzers "Pointe-180" ("Scientific", USA) were used with appropriate sets.

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manuals [16,18,22]. 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 circulating immune complexes (CIC, polyethylene glycol precipitation method) and Immunoglobulins classes M, G, A (ELISA, analyser "Immunochem", USA). In addition, the saliva level of secretory IgA, IgA and IgG was determined as well as Lysozime (by bacteriolysis of *Micrococcus lysodeikticus*). We calculated also the Entropy of Immunocytogram and Leukocytogram. Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [4] with moderately modification by MM Kovbasnyuk [17,24].

In addition, the blood level of cytokines IL-1, IL-6 and TNF- α as well as C-Reactive Protein was determined (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Diactone", France).

The condition of Microbiota is evaluated on the results of sowing of feces and urine.

Results processed by using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

According to calculations by the formula:

$$|r| = \frac{\exp[2t/(n-1,5)^{0,5}] - 1}{\exp[2t/(n-1,5)^{0,5}] + 1}$$

for a sample of n=44 critical value |r| at p<0,05 (t>2,02) is 0,30, at p<0,02 (t>2,42) is 0,35, at p<0,01 (t>2,70) is 0,39, at p<0,001 (t>3,55) is 0,50.

Based on the results of the screening, a correlation matrix is created (Table 1). For comparison, we present the correlation matrix from the previous article [9]. We draw attention to the difference between the critical levels of the coefficient modules, which is caused by twice the difference of the samples.

Table 1. Correlation matrix for changes in parameters of Uric Acid metabolism and Immunity

Variables	N=44; 0,05 r ≥0,30		N=88; 0,05 r ≥0,21		
	Change in UA Serum	Change in UA Excretion	UA Serum raw	UA Serum standard	UA Excretion
Uric Acid Serum	1,00	-,05	1,00	,87	-,03
UAS standardized by sex&age			,87	1,00	-,09
Uric Acid Excretion	-,05	1,00	-,03	-,09	1,00
CD4 ⁺ Lymphocytes	-,49	,29	-,38	-,13	-,23
IgA Saliva	-,36	,11	-,34	-,10	-,27
IgG Saliva	-,25	,04	-,27	,11	,13
Tumor Necrosis Factor- α	-,31	,08	,03	,03	,03
IL-6	-,31	,08	,03	,03	,03
C-Reactive Protein	-,31	,08	,03	,03	,03
Monocytes	,34	-,07	,27	,02	,20
Circulating Immune Complexes	,33	,04	,13	,17	-,01
CD8 ⁺ Lymphocytes	,26	-,17	,14	,25	,02
Microbial Count vs Staph. aureus	-,06	-,28	,21	,20	-,09
IgG Serum	,06	,23	,00	,01	-,07

First of all, it is found that individual **changes** in uricemia correlate inversely with **changes** in the level of T-helpers in blood (Fig. 1) as well as IgA and IgG in saliva while positively with **changes** in the level of Monocytes with the almost same force as their **static** levels.

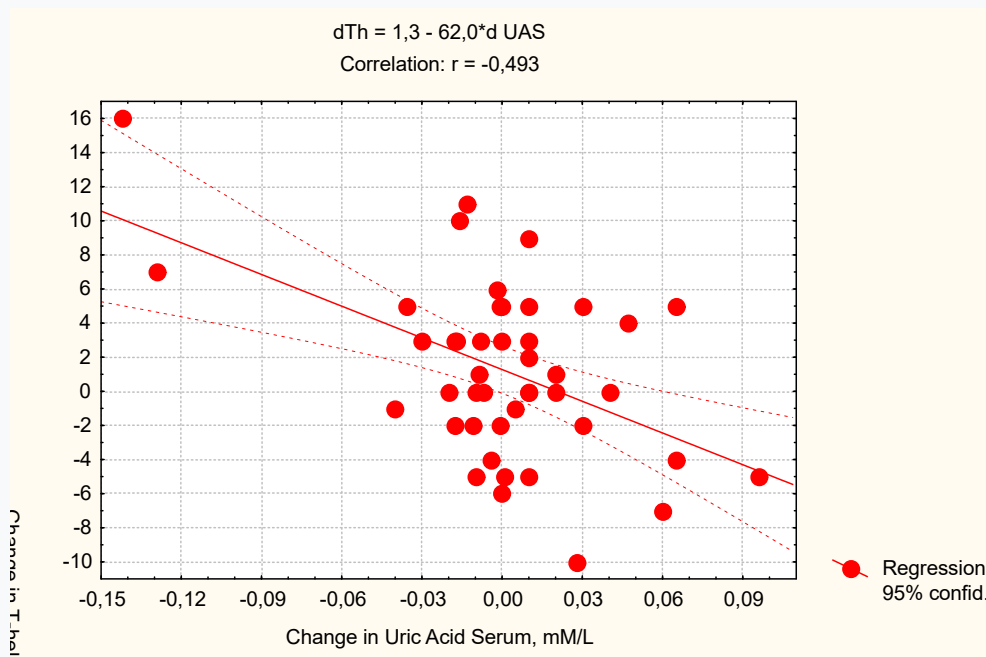


Fig. 1. Scatterplot of correlation between changes in Uric Acid Serum (X-line) and T-helpers (Y-line)

Instead, inverse links with changes in inflammatory markers and positive connections with changes in CIC and T-killers that are absent with respect to static parameters are revealed.

However, no association was found between changes in uricemia and the intensity of *Staph. aureus* phagocytosis by blood neutrophils.

Changes in uricosuria correlate with changes in only three immune parameters, and even at the limit of significance. Note the opposite signs of the correlation coefficients for T-helpers and T-killers, on the one hand, and uricemia and uricosuria, on the other. This is due to the inverse nature of the association between changes in the blood content of these subpopulations of T lymphocytes (Fig. 2).

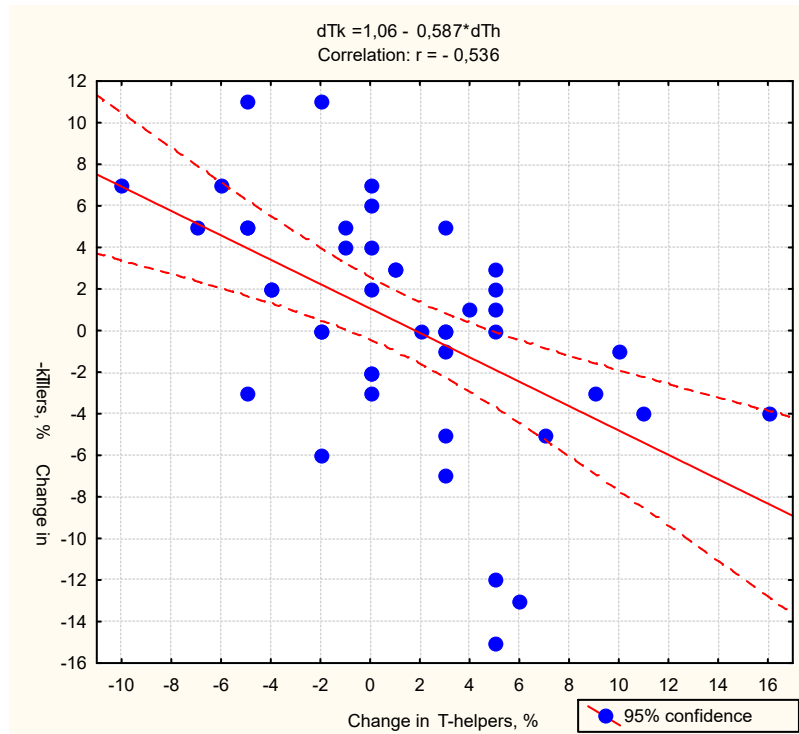


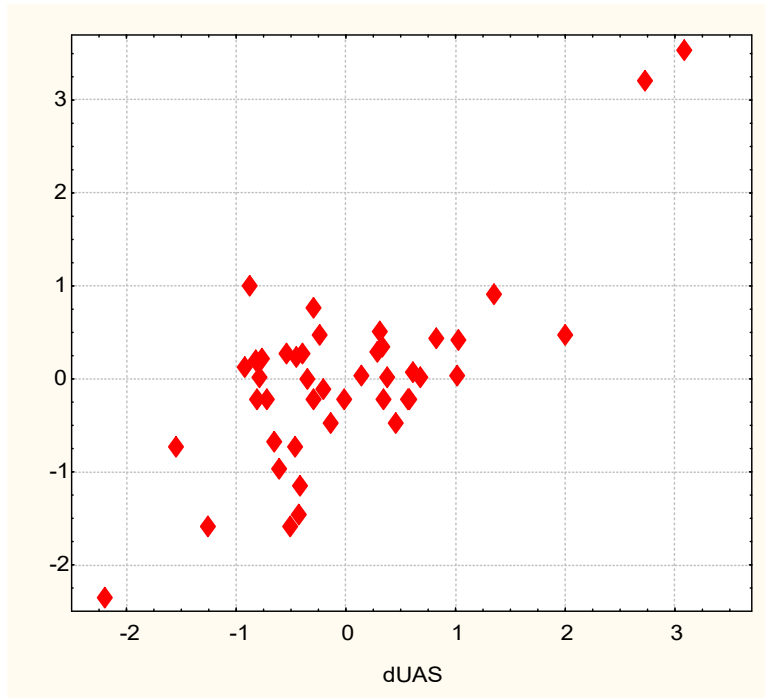
Fig. 2. Scatterplot of correlation between changes in T-helpers (X-line) and T-killers (Y-line)

By stepwise exclusion, 4 Immunity parameters were included in the regression model for change in uricemia, while some 3 parameters with significant coefficients were found outside the model. Such constellation of change in parameters of Immunity is determined by change in uricemia by 50% (Table 2 and Fig. 3).

Table 2. Regression Summary for change in Serum Uric Acid level

$R=0,740$; $R^2=0,547$; Adjusted $R^2=0,500$; $F_{(4,4)}=11,8$; $p<10^{-5}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(39)}$	p-level
Change in	r		Intercept	,0013	,0047	,27	,788
T-helpers	-0,49	-,420	,11	-,0033	,0009	-3,70	,001
TNF- α	-0,31	-,249	,11	-,0057	,0026	-2,17	,036
Monocytes	0,34	,386	,11	,0069	,0019	3,56	,001
CIC	0,33	,399	,11	,0008	,0002	3,66	,001



$R=0,740$; $R^2=0,547$; $\chi^2_{(4)}=32$; $p<10^{-5}$; Λ Prime=0,453

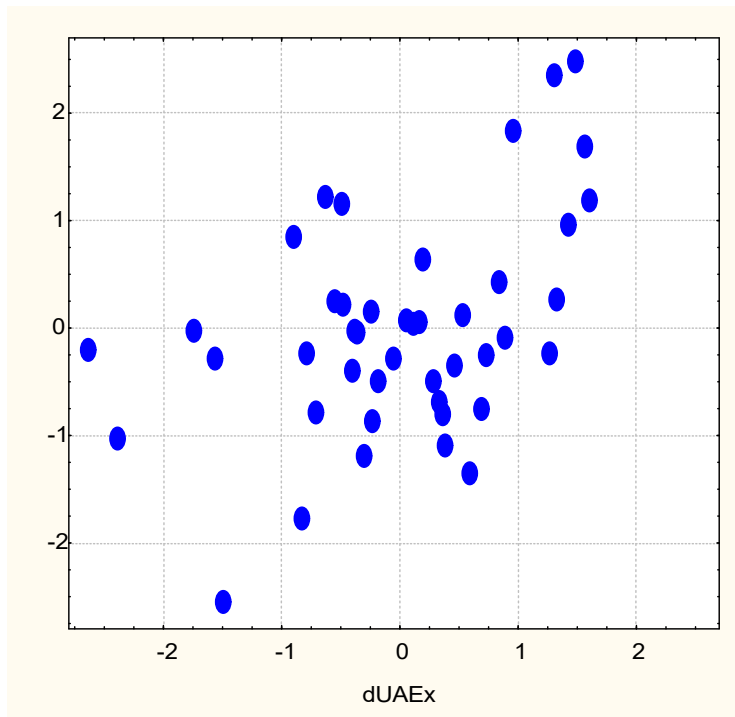
Fig. 3. Scatterplot of canonical correlation between changes in Uric Acid Serum (X-line) and Immunity parameters (Y-line)

Instead, change in uricosuria slightly determines only three Immunity parameters and is only 15,2% but statistically significant (Table 3 and Fig. 4).

Table 3. Regression Summary for change in Uric Acid Excretion

$R=0,459$; $R^2=0,211$; Adjusted $R^2=0,152$; $F_{(3,4)}=3,6$; $p=0,022$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(40)}$	p-level
Change in	r		Intercept	,017	,252	,07	,948
T-helpers	0,29	,284	,146	,095	,049	1,94	,059
IgG Serum	0,23	,177	,145	,070	,057	1,22	,230
MC St. aur.	-0,28	-,319	,142	-,060	,026	-2,25	,030



$R=0,459$; $R^2=0,211$; $\chi^2_{(3)}=9,6$; $p=0,022$; $\Lambda \text{ Prime}=0,789$

Fig. 4. Scatterplot of canonical correlation between changes in Uric Acid Excretion (X-line) and Immunity parameters (Y-line)

For the sake of maximum correlation, the link between changes in uric acid metabolism and $CD4^+$ T-helper lymphocytes is a top priority. It is appropriate to mention the classics here.

S Limatibul, A Shore, HM Dosch and EW Gelfand [19] three subpopulation of E-rosetting T-lymphocytes have been delineated: theophylline-sensitive T-cells (T_S) which lose the capacity to form E-rosette following treatment; theophylline-resistant T-cells (T_R) which are unaffected by the drug; and theophylline-dependent cells which acquire the ability to form E-rosettes following incubation with theophylline. The action of theophylline was shown to be dose-dependent (10^{-5} ÷ 10 mM/L). For immunoassays, concentrations of 1÷5 mM/L are used. T_R lymphocytes are $RFc\mu$ -enriched, $RFc\gamma$ -depleted and function as inducers of B lymphocytes differentiation. In contrast, T_S cells, are $RFc\gamma$ -enriched, $RFc\mu$ -depleted and suppress B lymphocytes differentiation.

RE Birch, AK Rosenthal and SH Polmer [2] first shown that Adenosine (0,01 mM/L) treatment did result in changes in OKT4 and OKT8 reactivity within the T_R subset. OKT4 expression was decreased from 71,8% to 58,3% after Adenosine treatment while percentage of OKT8 reactive cells increased from 16,5% to 33,0%. The two-fold increase in OKT8 expression is approximately equal in magnitude to the change observed in $RFc\gamma$ expression under the same conditions. There was only a small change in reactivity to OKT4 in T_{total} fraction after Adenosine treatment, however, significantly increased percentage of $OKT8^+$ cells were seen. According to the authors, the T_R cells expressing OKT8 after Adenosine treatment are likely to come, at least in part, from T_R cells which were OKT4. This is suggested by the fact that the sum of the $OKT4^+$ and $OKT8^+$ T_R cells remains constant before and after Adenosine treatment. The authors brilliantly speculated that expression of T lymphocytes $Fc\gamma$ receptors are regulated by agents acting upon Adenosine receptors.

It is already known that the immunotropic effect of Adenosine is realized through its receptors (A_1 , A_{2A} , A_{2B} , A_3), which express virtually all populations of immunocytes: T, NK, B lymphocytes, macrophages, neutrophils, dendritic and endothelial cells [1,12,13,27].

Theophylline (**2,6-dioxi-1,3-dimethylpurine** or 1,3-dimethylxantine) is a structural homolog of Adenosine [(2R,3R,4R,5R)-2-(6-aminopurine-yl)-5-(hydroxymethyl) oxolan-3,4-diol] and capable of 0,2 mM/L at blocking adenosine A_1 - i A_{2A} receptors[25].

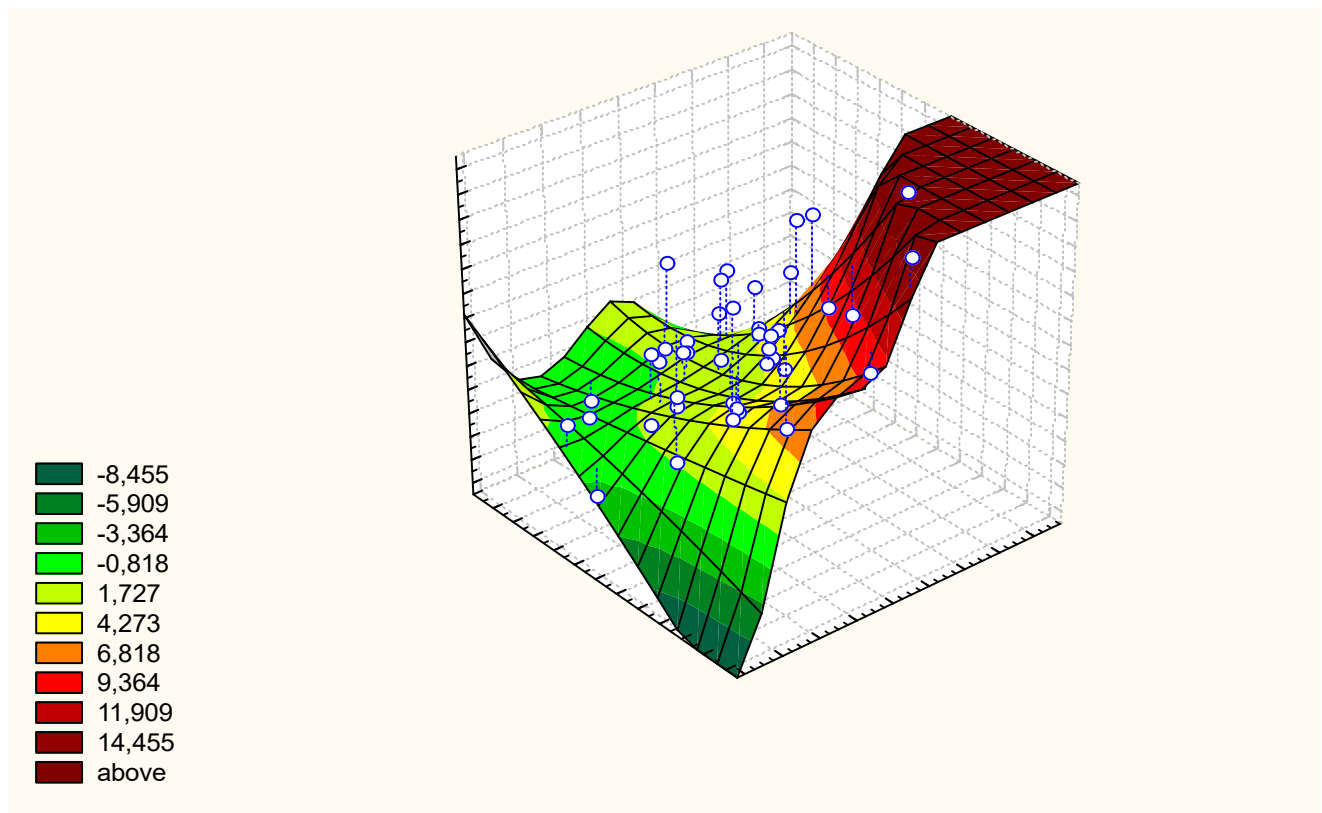
A non-selective Adenosine receptor antagonists, mainly A_{2A} , are caffeine (**2,6-dioxi-1,3,7-trimethylpurine** or 1,3,7-trimethylxantine) and other methylxanthines [20,21] which are introduced into the human body almost daily from coffee, tea and cocoa.

We hypothesized [6,15] that Uric acid (**2,6,8-trioxipurine**) is an **endogenous** non-selective Adenosine receptor antagonist.

However, the facts obtained in this and previous [9] studies on the inversely relationship of uricemia (the level of which is comparable to the concentrations of adenosine and theophylline in immune tests in vitro) with a relative blood content of $CD4^+CD3^+$ T-helper cells in combination with the presence of directly connection with the content of $CD8^+CD3^+$ T-cytolytic cells indicate the similarity of the effects of Uric acid with those of Adenosine, ie activation of A_{2A} receptors.

What about the opposite immunotropic effects of uricosuria?

Therefore, the question of the nature of the effect of Uric acid on immunocytes, in particular T-helper cells, remains open. Only the **immunomodulation** illustrated in Figure 5 can be claimed.



$$dTh(\%) = 1,28 - 60,18 \cdot dUAS(mM/L) + 0,874 \cdot dUAE(mM/24h)$$

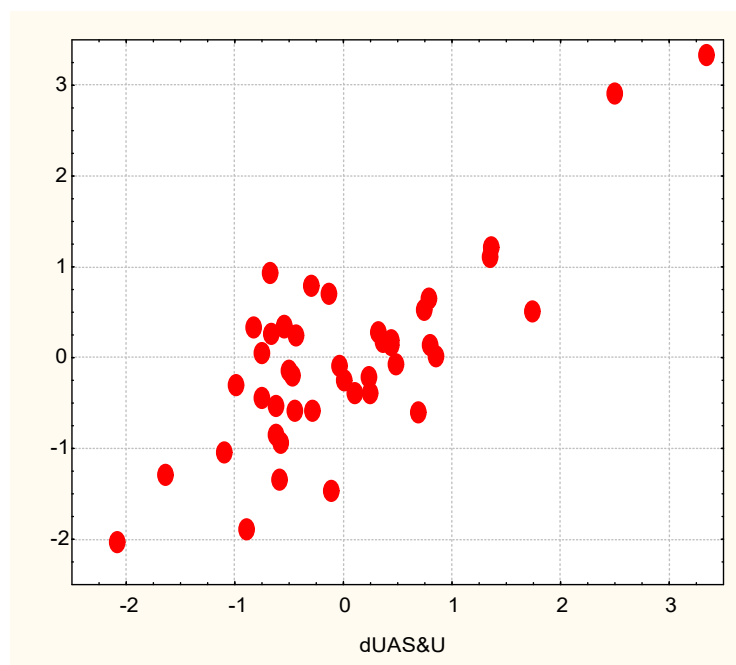
$$R=0,582; R^2=0,338; \text{Adjusted } R^2=0,305; F_{(2,4)}=10,2; p=0,0003$$

Fig. 5. Scatterplot of correlation between changes in Uric Acid Excretion (X-line), Uric Acid Serum (Y-line) and T-helpers blood level (Z-line)

Taking into account other relationships, we find that balneotherapy-induced changes in uricemia and uricosuria determine changes in immune status by 60% (Table 6 and Fig. 6).

Table 6. Factor structure of canonical correlation between changes in parameters of Uric Acid exchange and parameters of Immunity

<i>Right set</i>	R
Uric Acid Serum	-,945
Uric Acid Excretion	,374
<i>Left set</i>	R
T-helpers	,716
TNF- α	,408
Monocytes	-,440
CIC	-,379
Microbial Count St. aur.	-,050
IgG Serum	,025



$R=0,772$; $R^2=0,596$; $\chi^2_{(12)}=42$; $p<10^{-4}$; Λ Prime=0,334

Fig. 6. Scatterplot of canonical correlation between changes in Uric Acid Exchange parameters (X-line) and Immunity parameters (Y-line)

Despite expectations, no significant consideration was found regarding microbiota parameters. We explain this too short observation period (10 days). However, the hope is that there are negative links with inflammatory markers.

ACKNOWLEDGMENT

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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