RELATIONSHIPS BETWEEN PARAMETERS OF URIC ACID EXCHANGE AND IMMUNITY AS WELL AS MICROBIOTA IN PATIENTS WITH NEUROENDOCRINE-IMMUNE COMPLEX DYSFUNCTION

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Abstract

Background. Previously, we found a wide range of uric acid exchange parameters and functional relationships of uricemia and uricosuria with the parameters of immunity in healthy rats analyzed. The purpose of this study is to clarify such relationships in patients with neuroendocrine-immune complex dysfunction on the background of chronic pyelonephritis combined with cholecystitis in remission. **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. 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**. The canonical correlation analysis revealed that raw uricemia determines by 28% nine parameters of immunity (relative blood content of pan-lymphocytes and their CD4^{+}-, CD56^{+}-, 0-populations, relative content of polymorphonuclear neutrophils, intensity and completeness of their phagocytosis Staph. aureus and their bactericidal capacity, saliva content of IgG) as well as bacteriuria and content in E. coli feces. Uricemia, normalized by sex and age, determines by 25% another constellation of immunity parameters (relative CD8^{+}

lymphocytes content, CIC, E. coli phagocytosis intensity and completeness, Staph. aureus phagocytosis activity and completeness) 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%. **Conclusion.** Endogenous uric acid has a modulating overall beneficial effect on a number of immune and microbiota parameters in both healthy rats and people with neuroendocrine-immune complex dysfunction.

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

INTRODUCTION

Previously, we found a wide range of uric acid exchange parameters grouped into four clusters [7] and functional relationships of uricemia and uricosuria with the parameters of immunity in healthy rats analyzed [8-10].

According to the classic motto "Ex experimentum ad inhaero" ("From experiment to clinic"), the purpose of this study is to clarify such relationships in patients with neuroendocrine-immune complex dysfunction on the background of chronic pyelonephritis combined with cholecystitis in remission, documented in a previous study [22,23,29].

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) [22,23,25].

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 [6]. The analyzers "Pointe-180" ("Scientific", USA) were used with appropriate sets.

In portion of capillary blood we counted up Leukocytogram (LCG) (Eosinophils, Stub and Segmentonucleary Neutrophils, Lymphocytes and Monocytes) and calculated two variants of Adaptation Index as well as two variants of Strain Index by IL Popovych [1,20].

Strain Index-1 = $[(Eo/3,5-1)^2 + (SN/3,5-1)^2 + (Mon/5,5-1)^2 + (Leu/6-1)^2]/4$

Strain Index-2 = $[(Eo/2,75-1)^2 + (SN/4,25-1)^2 + (Mon/6-1)^2 + (Leu/5-1)^2]/4$

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manuals [13,16,19]. 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 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 (h) of Immunocytogram (ICG) and Leukocytogram (LCG) using formulas [21,24,30], adapted from classical CE Shannon's formula [27]:

$$hICG = - [CD4 \cdot \log_2 CD4 + CD8 \cdot \log_2 CD8 + CD22 \cdot \log_2 CD22 + CD56 \cdot \log_2 CD56]/\log_2 4$$

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

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [3] with moderately modification by MM Kovbasnyuk [15,26]. 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 [25]:

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

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

First of all, we consider it necessary to determine the standards of uric acid metabolism parameters. It is generally accepted that they are determined by sex and age, however, the specific values, according to different sources, do not match. In particular, differences between uricemia rates for different age groups are 5% for men and 21% for women, and gender differences range from 13-42% (see [12] and [14]).We have adopted the following uricemia standards for gender and age (Table 1).

Table 1. Uricemia standards for age and gender

Age	Serum Uric Acid, mM/L	
	Males	Females
23-29	0,375	0,290
30-39	0,390	0,275
40-49	0,392	0,278
50-59	0,388	0,305
60-69	0,385	0,333
>69	0,380	0,310

With regard to uricosuria standards, the situation is less ambiguous, as various authors do not consider gender differences to be significant. We have adopted as a standard the average of 3,0 mM/24h (range 1,5÷4,5 mM/24h, which is very close to the average literary range of 1,80÷4,46 mM/24h [12]).

Correlation Links Screening has found, first, their complete absence between uricemia and uricosuria (Table 2). Second, raw uricemia levels are more closely related to Immunity and Microbiota parameters than levels standardized by sex and age. Third, uricosuria was significantly associated with only 5 parameters of immunity.

Table 2. Correlation matrix between parameters Uric acid exchange and Immunity as well as Microbiota (color-coded significant links)

N=88 $_{0,05}|r|\geq0,21$	UAS raw	UAS standard	UA Excretion
UAS raw	1,00	**,87**	-,03
UAS standardised by sex&age	**,87**	1,00	-,09
UA Excretion	-,03	-,09	1,00
CD4+ Lymphocytes	**-,38**	-,13	**-,23**
Killing Index vs E. coli	,38	,23	,11
Attenuated E. coli feces	**-,38**	**-,22**	-,12
Killing Index vs Staph. aureus	**,36**	**,24**	,16
Monocytes	**,27**	,02	,20
Bactericidal Capacity vs E. coli	**,26**	**,28**	,06
IgG Saliva	**-,27**	,11	,13
Lactobacillus feces	**,27**	,14	,09
Bifidobacter feces	**,25**	,12	,08
Polymorphonucleary Neutrophils	**-,22**	-,00	-,15
Entropy Immunocytogram	**,22**	,08	**,24**
Microbial Count vs Staph. aureus	**,21**	,20	-,09
Bacteriuria, lg CFU	**-,20**	**-,24**	-,02
Bacteriuria, points	**-,20**	**-,23**	-,01
Bactericidal Capacity vs Staph. aur.	,20	**,22**	,04
CD56+ Lymphocytes	,20	-,06	,17
Phagocytose Index vs Staph. aureus	,14	**,25**	**-,22**
CD8+ Lymphocytes	,14	**,25**	,02
Microbial Count vs E. coli	,12	**,22**	-,10
Phagocytose Index vs E. coli	,03	,16	**-,27**
Pan-Lymphocytes	,19	-,01	,10
E. coli feces	,19	,02	,05
Popovych Adaptation Index-1	-,18	-,13	-,08
0-Lymphocytes	,17	,00	-,01
IgA Serum	-,15	,07	-,18
Hemolytica E. coli feces	-,15	-,05	-,05
Active T-Lymphocytes	-,14	-,02	-,02
CIC Serum	,13	,17	-,01
Entropy Leukocytogram	,12	-,02	,17
Leukocyturia raw	-,12	,04	-,02
Klebsiela&Proteus feces	,04	,16	-,04
Leukocyturia points	,01	,12	,06
IgM Serum	,06	,06	-,16
CD22+ Lymphocytes	,02	,03	,16
Leukocytes Blood	,00	-,04	,16
Eosinophils	-,09	,03	,01
Stubnuclear Neutrophils	-,08	,01	-,03
Popovych Strain Index-2	-,06	,02	,01
Erythrocyturia points	,03	,08	-,01
Popovych Adaptation Index-2	-,03	-,02	-,11
TNF-α	,03	,03	,03
IL-6	,03	,03	,03
CRP	,03	,03	,03
IL-1	,02	,02	,07
IgG Serum	,00	,01	-,07
Erythrocyturia raw	-,02	,01	,04
Popovych Strain Index-1	-,01	,02	-,01

By stepwise exclusion, 9 Immunity parameters were included in the regression model for raw uricemia, as well as bacteriuria and E. coli content, despite very low correlation coefficients, while some parameters with significant coefficients were found outside the model. Such constellation of parameters of Immunity and Microbiota is determined by raw uricemia by 28% (Table 3 and Fig. 1).

Table 3. Regression Summary for Serum Uric Acid raw level
R=0,611; R^2=0,374; Adjusted R^2=0,283; $F_{(12)}$=4,1; $p<10^{-4}$

|		Beta	St. Err. of Beta	B	SE of B	$t_{(76)}$	p-level
Variables	r		Intercpt	1,533	,5238	2,93	,005
CD4+ Lymphocytes, %	-,38	-,299	,196	-,0031	,0021	-1,53	,130
Polymorphonucleary Neutrophils, %	-,22	-,394	,312	-,0040	,0032	-1,26	,211
Bacteriuria, points	-,20	-,336	,151	-,1094	,0492	-2,22	,029
Killing Index vs Staph. aureus, %	,36	,555	,153	,0053	,0015	3,62	,001
IgG Saliva, mg/L	-,27	-,217	,139	-,0075	,0048	-1,56	,122
Microbial Count vs Staph. aur, Bac/Phag	,21	,371	,130	,0037	,0013	2,86	,006
Bactericidal Capacity vs St. aur., 10^9 B/L	,20	-,397	,197	-,0013	,0007	-2,01	,048
CD56+ Lymphocytes, %	,20	-,233	,161	-,0030	,0021	-1,45	,152
Pan-Lymphocytes, %	,19	-,443	,341	-,0047	,0036	-1,30	,199
E. coli feces, lg CFU	,19	-,271	,147	-,0848	,0459	-1,85	,069
0-Lymphocytes, %	,17	,166	,134	,0024	,0020	1,24	,220
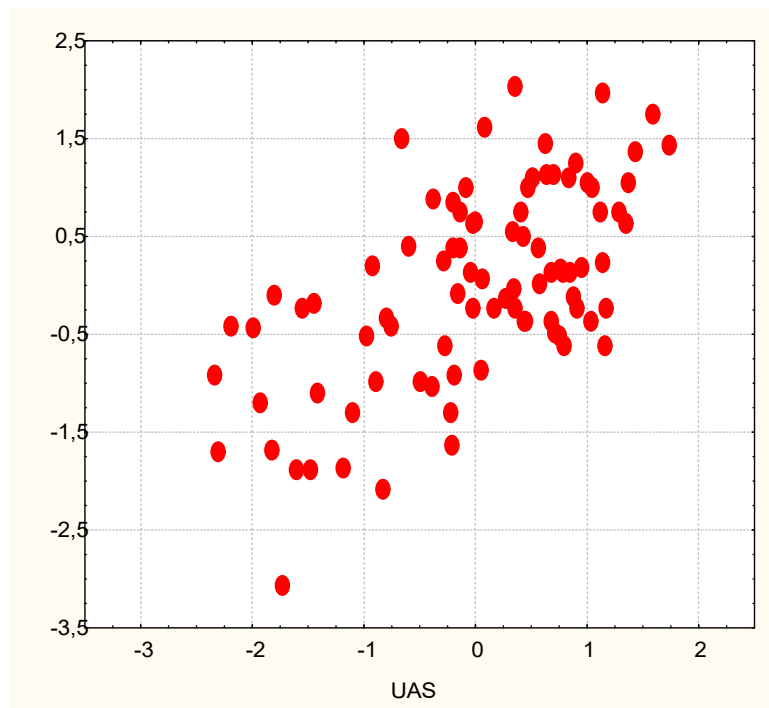
R=0,611; R^2=0,374; $\chi^2_{(11)}$=38; $p<10^{-4}$; Λ Prime=0,626
Fig. 1. Scatterplot of canonical correlation between Serum Uric Acid raw level (X-line) and parameters of Immunity and Microbiota (Y-line)

Interestingly, uricemia standardized by sex and age determines another constellation of Immunity and Microbiota parameters, but at almost the same rate as the actual one (Table 4 and Fig. 2).

Table 4. Regression Summary for Serum Uric Acid level standardized by Sex and Age
R=0,565; R^2=0,319; Adjusted R^2=0,250; F$_{(8,8)}$=4,6; p=0,0001

	r	Beta	St. Err. of Beta	B	St. Err. of B	t$_{(79)}$	p-level
Variables			Intercpt	-23,6	10,3	-2,30	,024
CD8$^+$ Lymphocytes, %	,25	,159	,098	,036	,022	1,61	,111
Phagocytose Index vs Staph. aureus, %	,25	,232	,115	,221	,110	2,01	,048
Killing Index vs Staph. aureus, %	,24	,278	,132	,036	,017	2,10	,039
Killing Index vs E. coli, %	,23	-,476	,284	-,041	,025	-1,67	,098
Microbial Count vs E. coli, Bact/Phag	,22	,162	,121	,022	,016	1,34	,184
CIC Serum, units	,17	,137	,098	,010	,007	1,40	,164
Klebsiela&Proteus feces, %	,16	,346	,120	,026	,009	2,89	,005
Attenuated E. coli feces, %	-,22	-,698	,294	-,028	,012	-2,38	,020
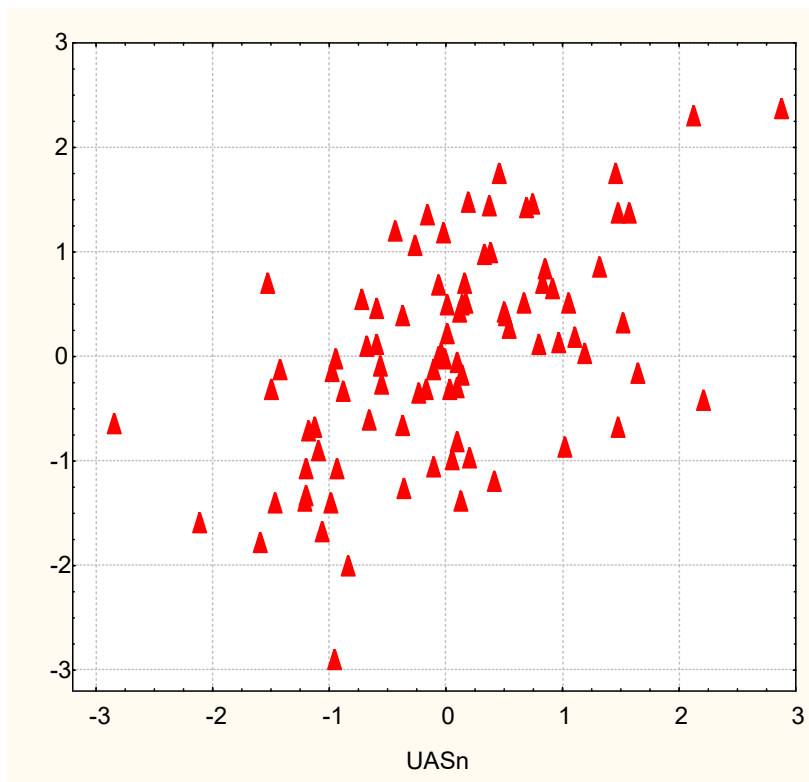
R=0,565; R^2=0,319; $\chi^2_{(8)}$=31,5; p=0,0001; Λ Prime=0,681

Fig. 2. Scatterplot of canonical correlation between Serum Uric Acid level standardized by sex and age (X-line) and parameters of Immunity and Microbiota (Y-line)

Instead, uricosuria slightly determines only four Immunity parameters and is only 11,5% but statistically significant (Table and Fig. 3).

Table 5. Regression Summary for Uric Acid Urinary Excretion
R=0,395; R^2=0,156; Adjusted R^2=0,115; F$_{(4,8)}$=3,8; p=0,007

|		Beta	St. Err. of Beta	B	St. Err. of B	t$_{(83)}$	p-level
Variables	r		Intercpt	12,63	19,01	,66	,508
Phagocytose Index vs E. coli	**-,27**	-,203	,105	-,30	,15	-1,94	,056
IgM Serum	-,16	-,139	,103	-,94	,69	-1,35	,179
Entropy Immunocytogram	**,24**	,184	,104	13,89	7,87	1,77	,081
Entropy Leukocytogram	,17	,198	,102	9,44	4,85	1,95	,055
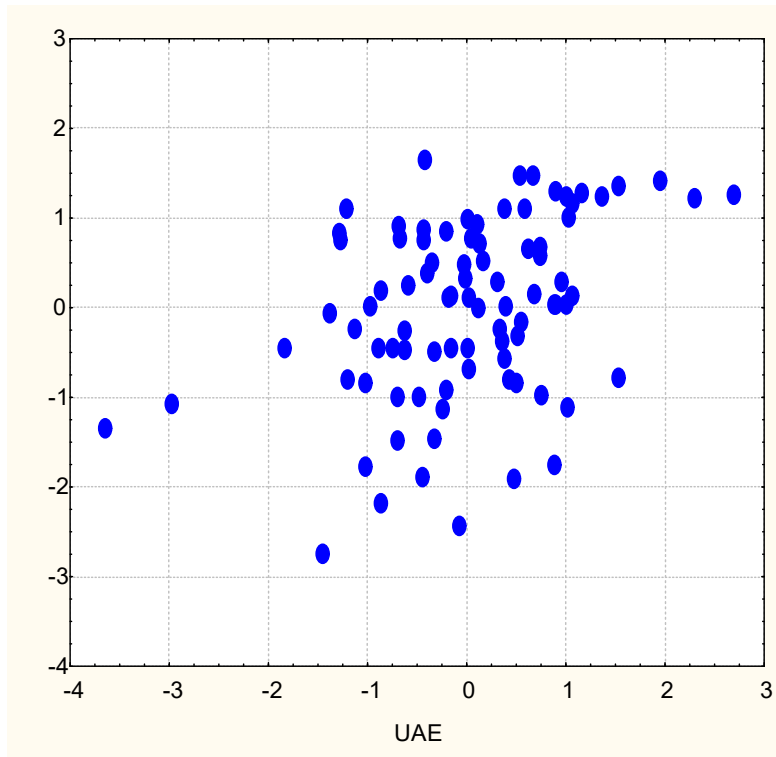
R=0,395; R^2=0,156; $\chi^2_{(4)}$=14,2; p=0,007; Λ Prime=0,844

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

In the final stage of the analysis, the relationship between the three parameters of Uric acid metabolism, on the one hand, and the parameters of Immunity and Microbiota, on the other, was clarified. Taken together, they have a more significant effect on Immunity and the Microbiota than taken separately (Table 6 and Fig. 4).

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

Right set	R
Uric Acid Serum raw, mM/L	-,890
Uric Acid Serum standardized by sex&age, Z	-,599
Uric Acid Excretion, mM/24h	-,293
Left set	R
CD4^{+} Lymphocytes, %	,724
Attenuated E. coli feces, %	,593
Polymorphonucleary Neutrophils, %	,490
IgG Saliva, mg/L	,489
Phagocytose Index vs E. coli, %	,187
Bacteriuria, points	,187
Klebsiela&Proteus feces, %	,072
Phagocytose Index vs Staph. aureus, %	,038
Killing Index vs E. coli, %	-,581
Killing Index vs Staph. aureus, %	-,556
CD56^{+} Lymphocytes, %	-,504
Entropy Immunocytogram	-,457
Pan-Lymphocytes, %	-,421
E. coli feces, lgCFU	-,370
0-Lymphocytes, %	-,326
Entropy Leukocytogram	-,325
Bactericidal Capacity vs Staph. aur., 10^9 B/L	-,208
Microbial Count vs Staph. aur., Bact/Phagoc	-,202
CIC Serum, units	-,099
CD8^{+} Lymphocytes, %	-,060
IgM Serum, g/L	-,008
Microbial Count vs E. coli, Bacter/Phagocyte	-,003
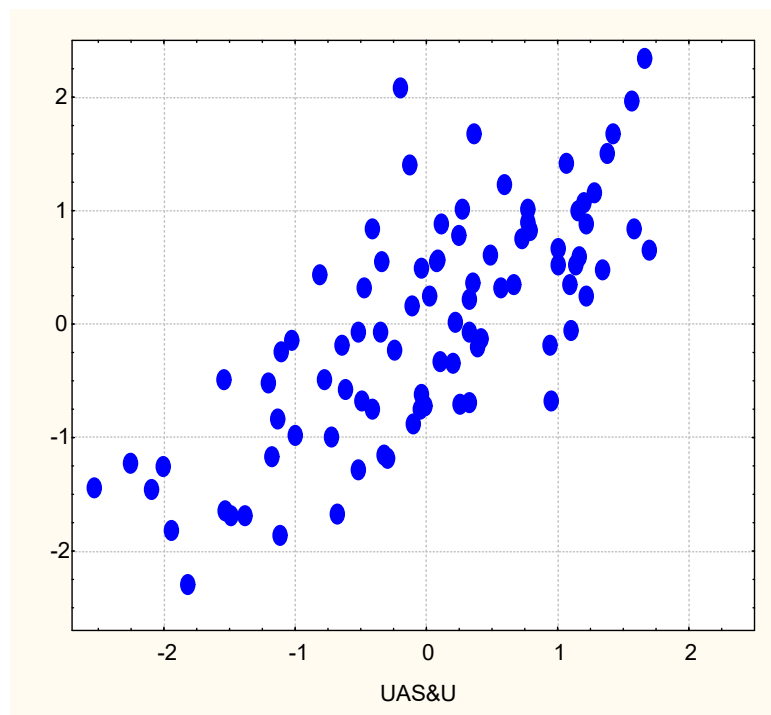
R=0,747; R^2=0,559; $\chi^2_{(66)}$=108; p=0,0008; Λ Prime=0,231

Fig. 4. Scatterplot of canonical correlation between parameters of Uric Acid exchange (X-line) and parameters of Immunity and Microbiota (Y-line)

Judging by factor loadings, the major targets of Uric acid suppressor activity are CD4^{+} Lymphocytes and Polymorphonucleary Neutrophils levels in the blood as well as IgG levels in saliva, to a lesser extent E. coli et Staph. aureus Phagocytosis Activity. Instead, the impact on both the Intensity and, in particular, the Completion of Phagocytosis by neutrophils of both bacterial species, blood levels of Natural and T-killers, as well as serum levels of CIC and IgM is enhancing.

In rats [8,10], we found that Uric acid level correlated positively with both the Activity and Intensity of Staph. aureus phagocytosis, while negatively with the blood levels of Natural Killer cells.

In this line we found only one work [2], which shows that incubation with Uric acid inhibits killing activity of neutrophil-like cells (HL-60) against Pseudomonas aeruginosa, while increased super oxide anion production. Another study [5] states that male hyperuricemia patients display a lower number of NK cells before and after a low-purine diet.

It is known that the level in the blood of immunocytes is a consequence of the interaction of three processes. With regard to CD4^{+} Lymphocytes and Polymorphonucleary Neutrophils, this is, firstly, a decrease in the flow of cells into the blood from the thymus and bone marrow, respectively; second, increased cell migration from blood to the spleen, lymph nodes, etc.; third, the activation of cell apoptosis.

In the above experiment in rats [8-10], we found that Uric acid level correlated positively with the content in the thymus of Lymphocytes and Macrophages, but negatively correlated with the content of Epitheliocytes, Reticulocytes and Hassal's corpuscles. If these data are transferred to humans, it can be suggested that Uric acid stimulates the differentiation of thymocytes to CD8^{+} T-cells, instead reducing the formation of CD4^{+} T-cells.

In line with the concept we have previously adopted [7] that Uric acid is structural homolog of Caffeine, which in turn is a structural homolog of Adenosine [17,28], of particular interest are the data that peripheral T-cell depletion due to inhibition of T-cell expansion by extracellular Adenosine mediated signaling through A_{2A} receptors [11]. It is shown that Caffeine increases cell death and migration in Cytotoxic but not Helper T-Lymphocytes as well as B-Lymphocytes in familiar but not naïve individuals following moderate intensity exercise [18].

As for the negative correlation of Uric acid and Polymorphonucleary Neutrophils levels, we have no reason to speculate. They may migrate to the spleen and/or get blocked in the bone marrow.

For the first time in our experiments and in the clinic, the effect of Uric acid on the Entropy of immunocytes was studied. In particular, the pro-entropic effect on the blood Immunocytogram was ascertained in both humans and rats [8,10], instead of Leukocytogram only in humans. In rats, Uric acid also has a neg-entropic effect on the Thymocytogram and Splenocytogram [8,10].

In general, we consider the influence of Uric acid on the parameters of Immunity favorable, except for the reduction of T-helpers in blood and IgG in saliva. And in that. it is known that low plasma Uric acid level associated with autoimmune inflammatory diseases [4].

The positive conclusion is evidenced by the data on the decrease of Bacteriuria, as well as the content in the feces of Klebsiela&Proteus as well as Attenuated E. coli in combination with the increase in the content of ordinary E. coli.

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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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