Kuchma Igor L., Gozhenko Anatoliy I., Bilas Volodymyra R., Ruzhylo Sofiya V., Kovalchuk Galyna Y., Nahurna Yaryna V., Zukow Walery, Popovych Igor L. Immunotropic effects of nitrogenous metabolites (creatinine, urea, uric acid and bilirubin) in humans exposed to the factors of the accident at the Chornobyl nuclear power plant. Journal of Education, Health and Sport. 2020;10(12):314-331. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2020.10.12.031
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.12.031
https://zenodo.org/record/4641198

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 09.12.2020. Revised: 25.12.2020. Accepted: 30.12.2020.

IMMUNOTROPIC EFFECTS OF NITROGENOUS METABOLITES (CREATININE, UREA, URIC ACID AND BILIRUBIN) IN HUMANS EXPOSED TO THE FACTORS OF THE ACCIDENT AT THE CHORNOBYL NUCLEAR POWER PLANT

Igor L. Kuchma[1], Anatoliy I. Gozhenko[1], Volodymyra R. Bilas[5], Sofiya V. Ruzhylo[2], Galyna Y. Kovalchuk[2], Yaryna V. Nahurna[3], Walery Zukow[4], Igor L. Popovych[1,5]

[1]Ukrainian Scientific Research Institute for Medicine of Transport, Odesa, Ukraine igorkuchma@ukr.net; prof.gozhenko@gmail.com
[2]Ivan Franko Pedagogical University, Drohobych, Ukraine doctor-0701@ukr.net
[3]Danylo Halyts'kyĭ National Medical University, L'viv, Ukraine nyv86@ukr.net
[4]Nicolaus Copernicus University, Torun, Poland w.zukow@wp.pl
[5]Bohomolets' OO Institute of Physiology of National Academy of Sciences, Kyïv, Ukraine i.popovych@biph.kiev.ua

Abstract

Background. In previous studies, we found that nitrogenous metabolites exhibit significant immunotropic activity, both suppressor and enhancing, at healthy rats. The **purpose** of this and our subsequent research is to elucidate the links between nitrogenous metabolites and immune parameters in different categories of people, both healthy and sick. **Material and methods**. The object of observation in 1997 were 19 men and 3 women who were exposed to pathogenic factors of the accident at the Chornobyl nuclear power plant during the liquidation of its consequences in 1986-87. The survey was conducted twice - on admission and after two weeks of rehabilitation at the Truskavets' Spa. The plasma and urinary concentration of the nitrogenous metabolites were determined. Immune status was assessed on tests of I and II levels according to the WHO memorandum. **Results**. Both negative and positive metabolic-immune correlations were revealed. Calculation of multiple correlation coefficients between individual metabolite parameters and constellations of immune parameters revealed the maximum immunotropic effect of Urea urine (R=0,756). This is followed by Uric acid plasma (R=0,727) and urine (R=0,691), Urea plasma (R=0,622), Creatinine plasma (R=0,588), Bilirubinemia (R=0,546) and Creatinine urine (R=0,510). The canonical correlation between the constellation of nitrogenous metabolites, on the one hand, and the parameters of

immunity, on the other hand, was very strong: R=0,971; $\chi^2_{(15)}$=239; $p<10^{-5}$. **Conclusion**. Nitrogenous metabolites have immunomodulatory effects, both suppressor and enhancing, both in healthy rats and in humans exposed to pathogenic influences.
Key words: **urea, uric acid, creatinine, bilirubin, immunity, relationships, humans.**

INTRODUCTION

In previous studies, we found significant links between nitrogenous metabolites of plasma and urine, on the one hand, and immune parameters, on the other hand, in healthy rats [32]. We also found the immunotropic effect of uric acid both in healthy rats [14,15] and patients of Truskavets' Spa [16,17,35]. The **purpose** of this and our subsequent research is to elucidate the links between nitrogenous metabolites and immune parameters in different categories of people, both healthy and sick.

MATERIAL AND METHODS

The database of the Truskavetsian Scientific School of Balneology [22,31] was used for the research. The object of observation in 1997 were 19 men (26÷61 years) and 3 women (38, 40 and 47 years) with urate urolithiasis and chronic pyelonephritis who were exposed to pathogenic factors of the accident at the Chornobyl nuclear power plant during the liquidation of its consequences in 1986-87. According to the documents, the total effective radiation dose was 10÷25 cGy, which is most typical for this contingent [22,31,34]. The survey was conducted twice: on admission and after two weeks of rehabilitation at the Truskavets' Spa.

The plasma level of the nitrogenous metabolites determined: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method) and bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method). The same metabolites, with the exception of bilirubin, were also determined in the morning urine. The analyzes were carried out according to the instructions described in the manual [12]. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets.

In portion of capillary blood we counted up Leukocytes level, Leukocytogram and its entropy [30]. In the venous blood, the parameters of immunity were determined as described in the manual [28]. The state of cellular immunity judged by the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by Jondal M et al [21], their theophylline-resistant and theophylline-sensitive subpopulations (by the test of sensitivity of rosette formation to theophylline by Limatibul S et al [23] as well as subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. Additionally evaluated the transformation of T-lymphocytes into blasts under the influence of phytohemagglutinin. Natural killers were identified as large granules contain lymphocytes.

The state of humoral immunity judged by the relative content of the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by Bianco C [3], the concentration in serum circulating immune complexes (by polyethylene glycol precipitation method) and Immunoglobulins classes M, G, A (by single radial immunodiffusion method by Mancini G et al [24]) as well as γ-globulines and C-reactive protein.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by the phagocytosis index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [4,8]. In addition, the serum level of Lysozime (by the test of bacteriolysis of Micrococcus lysodeikticus) and Complement (by 50% hemolysis in the complement fixation reaction) was determined.

Digital material is statistically processed on a computer using the software package "Statistica 20".

RESULTS

In presenting the results, we will follow the algorithm used to analyze the experimental data [32].

Screening of linear correlation coefficients between parameters of nitric metabolites, on the one hand, and the recorded parameters of immunity, on the other hand, revealed the following (Table 1). The table does not include registered immunity parameters, which did not reveal any noteworthy correlation coefficient with nitrogenous metabolites.

In the next step of the analysis, a regression model was constructed for each plasma and urine nitrogenous metabolite by stepwise exclusion until the maximum level of adjusted R^2 was reached. As a result, it turned out that some regression models **included** parameters with an insignificant correlation coefficient, while some parameters with a significant correlation were outside the model.

Table 1. Matrix of correlations between Nitrogenous metabolites and parameters of Immunity

N=44 $_{0,05}\|r\|\geq 0,30$; $_{0,02}\|r\|\geq 0,36$; $_{0,01}\|r\|\geq 0,39$; $_{0,001}\|r\|\geq 0,50$	Urea Urine	Uric acid Urine	Creati-nine Urine	Urea Plas-ma	Crea-tinin-emia	Uric-emia	Bili-rubin-emia
Phagocytose Index of Neutrophils, %	-0,13	0,01	**-0,29**	0,29	**0,24**	-0,03	**0,23**
Microbial Count of Neutrophils, Bact/Phagoc	-0,02	0,03	-0,13	-0,01	0,01	**-0,18**	-0,11
Killing Index of Neutrophils, %	0,11	0,16	**-0,22**	0,01	-0,05	-0,04	0,03
Bactericidal Capacity of Neutrophils, 10^9 B/L	0,08	0,20	-0,25	0,06	-0,04	-0,15	0,07
Phagocytose Index of Monocytes, %	-0,02	0,09	0,07	**0,26**	0,09	-0,03	0,02
Microbial Count of Monocytes, Bact/Phagoc	0,00	0,07	-0,18	-0,01	-0,00	-0,18	-0,10
Bactericidal Capacity of Monocytes, 10^9 B/L	0,24	**0,22**	0,08	-0,03	-0,26	-0,08	-0,15
Monocytes, 10^9/L	0,26	0,08	0,20	-0,13	**-0,30**	0,15	-0,20
Monocytes, %	0,26	0,04	**0,21**	-0,14	-0,29	0,14	-0,21
Polymorphonucleary Neutrophils, %	-0,16	-0,13	-0,06	-0,11	-0,16	-0,13	**0,23**
Eosinophils, %	**0,30**	0,18	0,10	-0,08	0,06	-0,12	-0,02
Pan-Lymphocytes, 10^9/L	0,02	0,17	0,02	**0,31**	**0,25**	**0,19**	-0,07
NK Lymphocytes, %	0,03	0,07	**0,23**	**-0,38**	**-0,38**	-0,04	0,19
Active T-Lymphocytes, %	0,02	0,21	0,08	-0,31	-0,20	-0,12	-0,08
Blast transformation of T-Lymphocytes, %	0,17	**0,25**	-0,00	**-0,37**	**-0,29**	0,04	-0,07
Theophylline-resistant T-Lymphocytes, %	-0,02	0,11	0,16	-0,11	-0,10	0,12	**-0,21**
Theophylline-sensitive T-Lymphocytes, %	**0,23**	-0,08	0,17	0,06	-0,03	**0,36**	0,02
B Lymphocytes, %	**0,36**	**0,55**	0,08	0,02	0,08	0,05	-0,05
Lysozyme, nM/L	0,19	0,17	-0,01	-0,21	-0,18	0,06	**-0,37**
IgA Serum, g/L	**-0,28**	-0,14	-0,25	-0,12	0,15	-0,11	0,08
IgM Serum, g/L	0,13	**-0,23**	0,18	0,13	0,08	**0,67**	-0,10
Complement, CH_{50}	**-0,32**	-0,34	-0,09	0,10	0,10	-0,04	0,06
C-Reactive Protein, points	**-0,45**	-0,11	-0,16	0,04	-0,06	-0,25	0,02
γ-globulines, g/L	**0,27**	0,28	0,19	-0,14	-0,10	0,01	0,10
γ-globulines, %	**0,23**	0,25	**0,28**	-0,11	-0,06	0,15	0,13

From the regression model it follows that bilirubinemia downregulates the Lysozyme activity (Fig. 1) and relative content in the blood of theophylline-resistant T-lymphocytes, while upregulates the content of Polymorphonucleary Neutrophils and its Phagocytose Index. Judging by adjusted R^2, the rate of immunomodulation is 30% (Table 2 and Fig. 2).

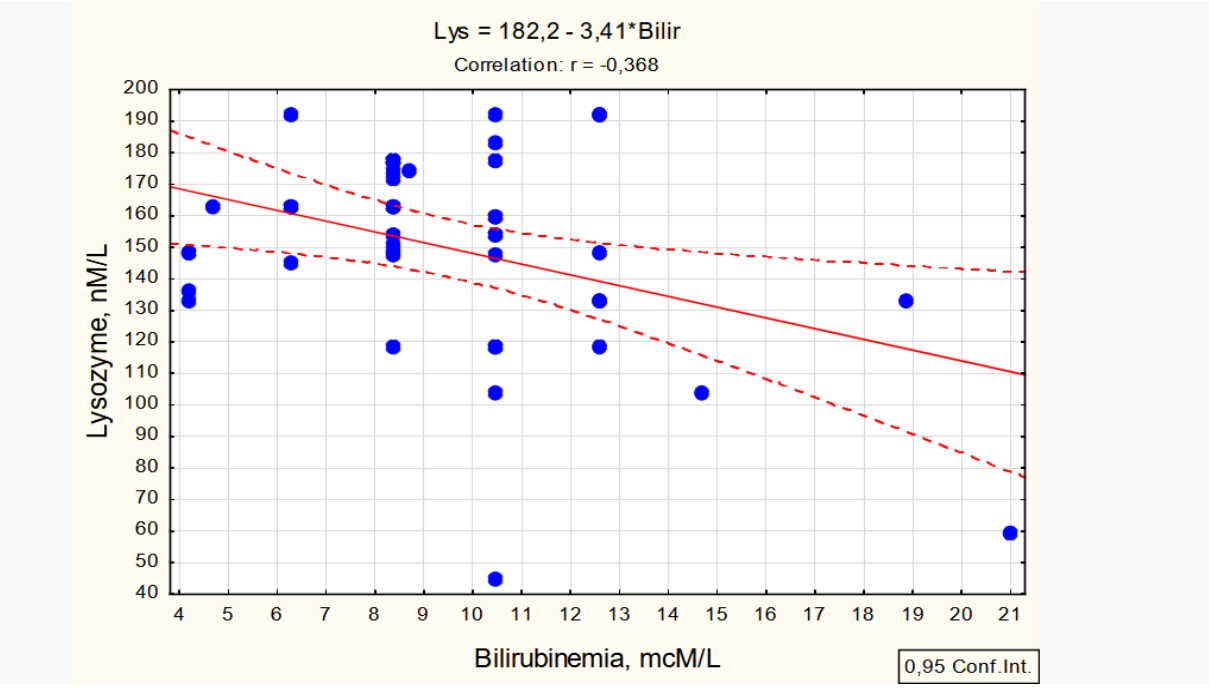
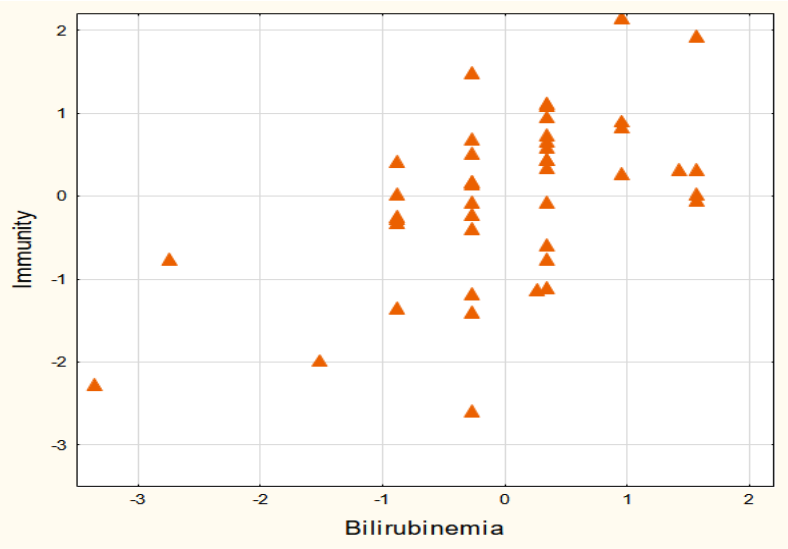


Fig. 1. Scatterplot of correlation between Bilirubinemia (X-line) and Lysozyme activity (Y-line)

Table 2. Regression Summary for Bilirubinemia
R=0,546; R^2=0,299; Adjusted R^2=0,227; $F_{(4,4)}$=4,2; p=0,007

		Beta	St. Err. of Beta	B	SE of B	$t_{(39)}$	p-level
Variables	r		Intercpt	0,887	5,404	0,16	0,870
Lysozyme, nM/L	-0,37	-0,537	0,178	-0,058	0,019	-3,01	0,005
Theophylline-resistant T-Lymphocytes, %	-0,21	0,194	0,179	0,104	0,096	1,08	0,287
Polymorphonucleary Neutrophils, %	0,23	0,293	0,138	0,145	0,069	2,12	0,040
Phagocytosis Index of Neutrophils, %	0,23	0,312	0,138	0,109	0,048	2,26	0,029



R=0,546; R^2=0,299; $\chi^2_{(4)}$=14,2; p=0,007; Λ Prime=0,701
Fig. 2. Scatterplot of canonical correlation between Bilirubinemia level (X-line) and parameters of Immunity (Y-line)

Uricemia upregulates the IgM serum level (Fig. 3) and relative content in the blood of theophylline-sensitive T-lymphocytes (Fig. 4) as well as pan-lymphocytes, while downregulates the C-reactive protein level and Microbial Count of Neutrophils. Such constellation of parameters of Immunity is determined by Uricemia by 53% (Table 3 and Fig. 5).

Table 3. Regression Summary for Uricemia, mM/L
$R=0,727$; $R^2=0,529$; Adjusted $R^2=0,467$; $F_{(5,4)}=8,5$; $p<10^{-4}$

		Beta	St. Err. of Beta	B	SE of B	$t_{(38)}$	p-level
Variables	r		Intercpt	-0,381	0,138	-2,75	0,009
IgM Serum, g/L	0,67	0,627	0,124	0,302	0,060	5,04	10^{-4}
Theophylline-sensitive T-Lymphocytes, %	0,36	0,216	0,117	0,005	0,003	1,84	0,074
Pan-Lymphocytes, 10^9/L	0,19	0,126	0,114	0,022	0,020	1,10	0,279
C-Reactive Protein, points	-0,25	-0,136	0,115	-0,015	0,012	-1,18	0,244
Microbial Count of Neutrophils, Bac/Phag	-0,18	0,153	0,124	0,010	0,008	1,24	0,223

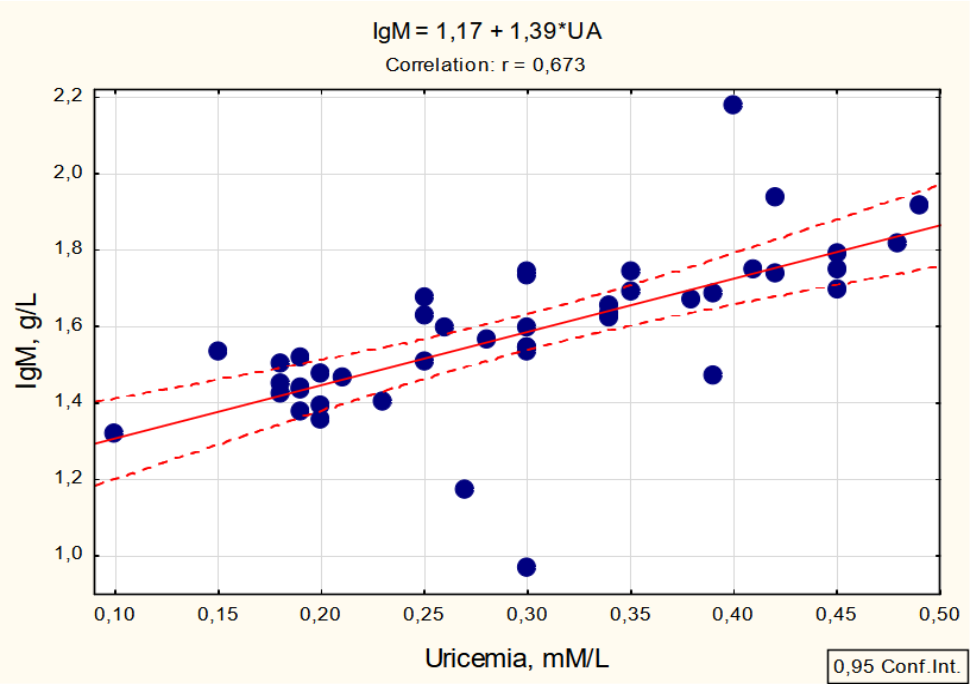


Fig. 3. Scatterplot of correlation between Uricemia (X-line) and IgM serum level (Y-line)

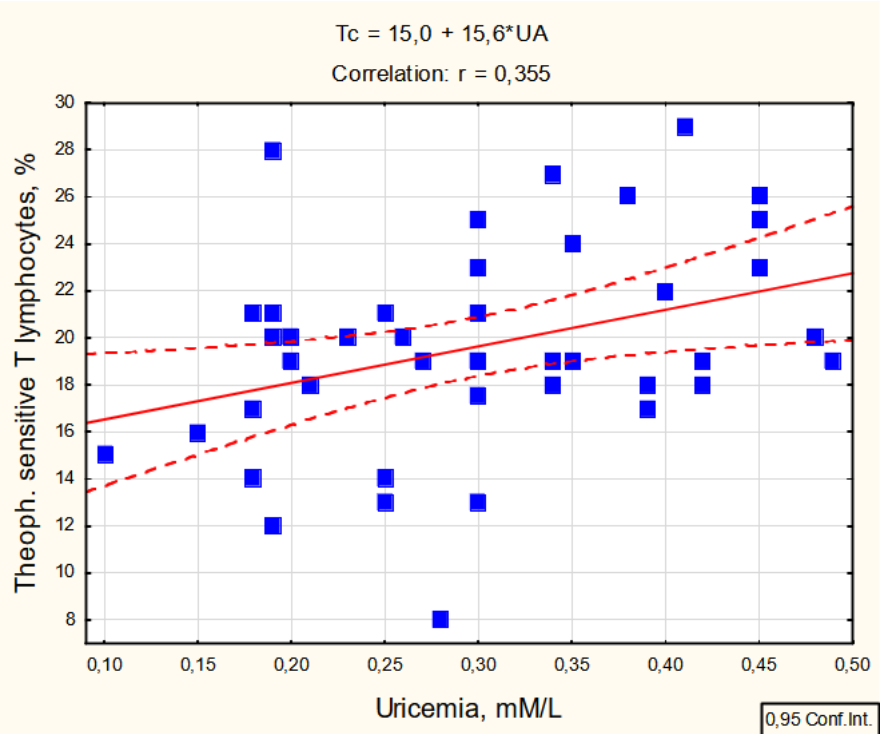
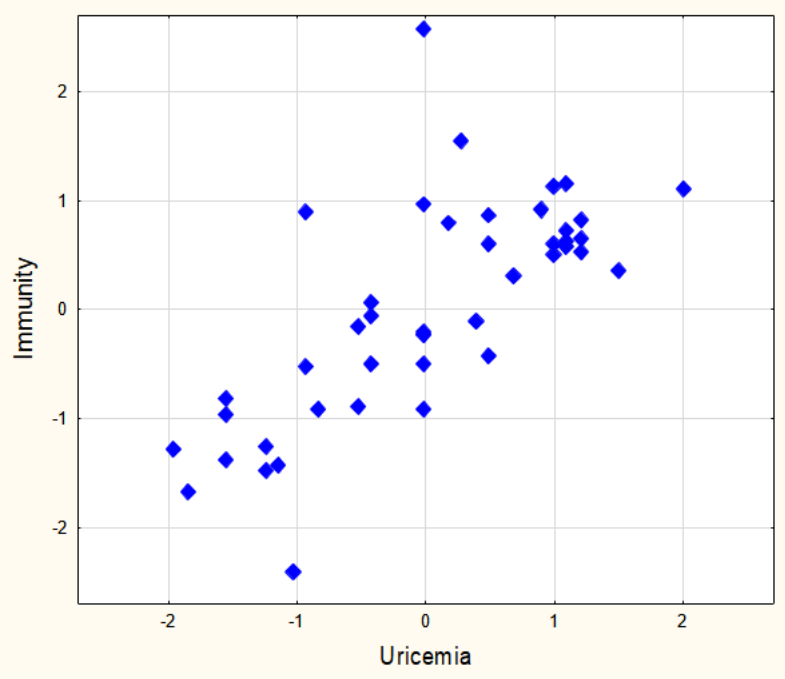


Fig. 4. Scatterplot of correlation between Uricemia (X-line) and theophyllin-sensitive T-lymphocytes level (Y-line)



R=0,727; R^2=0,529; $\chi^2_{(5)}$=29,8; p<10^{-4}; Λ Prime=0,471

Fig. 5. Scatterplot of canonical correlation between Uricemia level (X-line) and parameters of Immunity (Y-line)

Instead, the concentration of Uric acid in the urine correlates negatively with the level of IgM. On the other hand, it upregulates the relative content of B-lymphocytes in the blood (Fig. 6), the mitogenic ability of T-lymphocytes and the bactericidal ability of blood

monocytes. Such constellation of parameters of Immunity is determined by Uricosuria by 48% (Table 4 and Fig. 7).

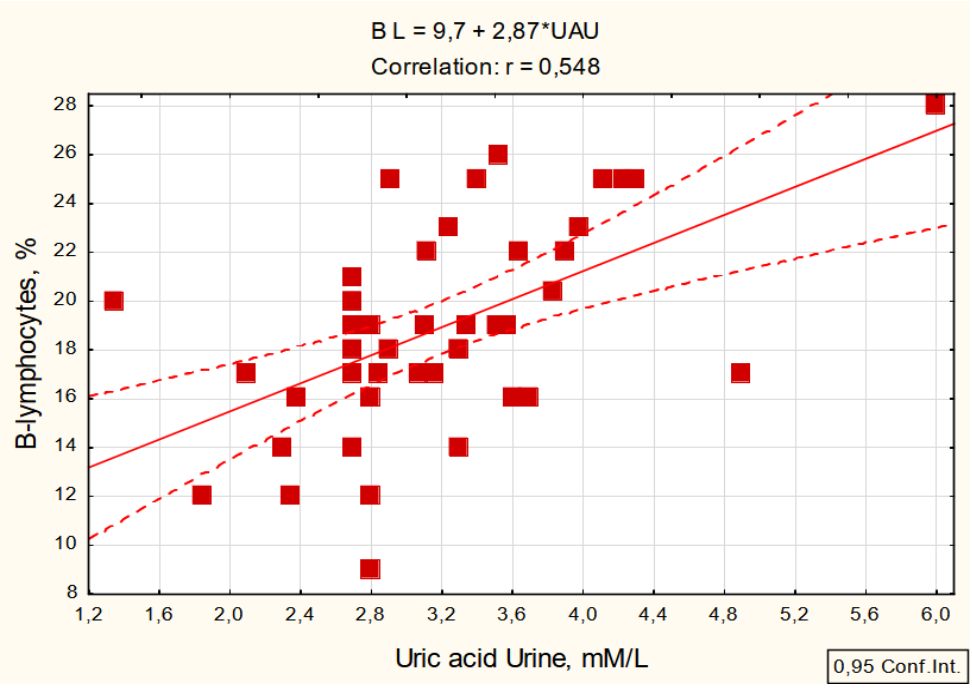
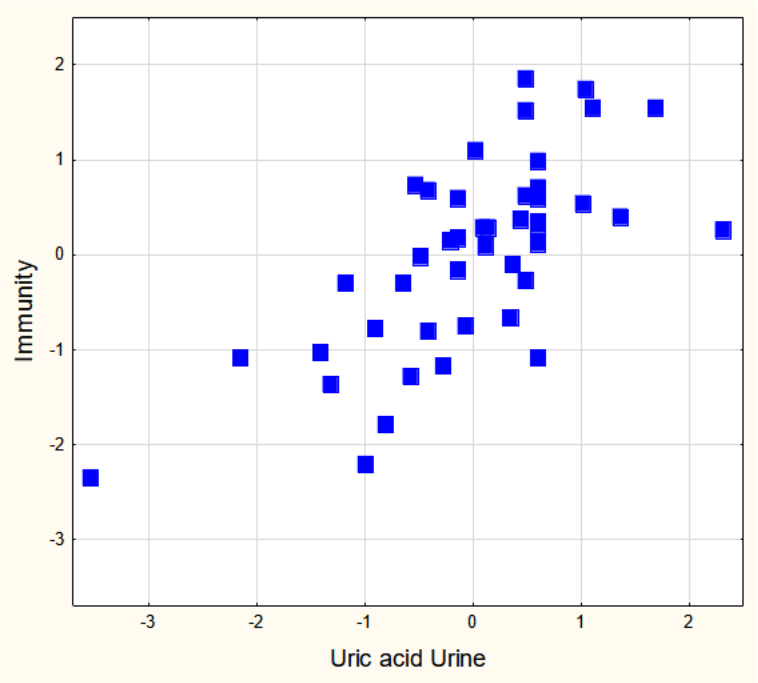


Fig. 6. Scatterplot of correlation between Uric acid Urine (X-line) and B lymphocytes level (Y-line)

Table 4. Regression Summary for Uric acid Urine, mM/L
R=0,691; R^2=0,477; Adjusted R^2=0,423; $F_{(4,4)}$=8,9; $p<10^{-4}$

		Beta	St. Err. of Beta	B	SE of B	$t_{(39)}$	p-level
Variables	r		Intercpt	1,455	0,947	1,49	0,144
B Lymphocytes, %	0,55	0,560	0,116	0,107	0,022	4,82	10^{-4}
Blast transformation of T-Lymphocytes, %	0,25	0,241	0,118	0,019	0,009	2,04	0,048
Bactericidal Capacity of Monocytes, 10^9 B/L	0,22	0,259	0,117	0,349	0,158	2,21	0,033
IgM Serum, g/L	-0,23	-0,233	0,117	-0,907	0,455	-1,99	0,053



R=0,691; R^2=0,477; $\chi^2_{(4)}$=25,9; $p<10^{-4}$; Λ Prime=0,523

Fig. 7. Scatterplot of canonical correlation between Uric acid Urine (X-line) and parameters of Immunity (Y-line)

Urea plasma level downregulates the relative content in the blood of NK-lymphocytes (Fig. 8) and the mitogenic ability of T-lymphocytes (Fig. 8), while upregulates the level of pan-lymphocytes and Microbial Count of Monocytes. Such constellation of parameters of Immunity is determined by Urea plasma by 39% (Table 5 and Fig. 9).

Table 5. Regression Summary for Urea Plasma, mM/L
R=0,622; R^2=0,387; Adjusted R^2=0,324; $F_{(4,4)}$=6,2; p=0,0006

		Beta	St. Err. of Beta	B	SE of B	$t_{(39)}$	p-level
Variables	r		Intercpt	5,462	1,432	3,82	0,0005
NK Lymphocytes, %	-0,38	-0,282	0,134	-0,131	0,062	-2,11	0,0418
Blast transformation of T-Lymphocytes, %	-0,37	-0,373	0,133	-0,057	0,020	-2,80	0,0079
Pan-Lymphocytes, 10^9/L	0,31	0,284	0,138	0,759	0,367	2,07	0,0454
Microbial Count of Monocytes, Bac/Phag	0,26	0,211	0,132	0,028	0,018	1,60	0,1187

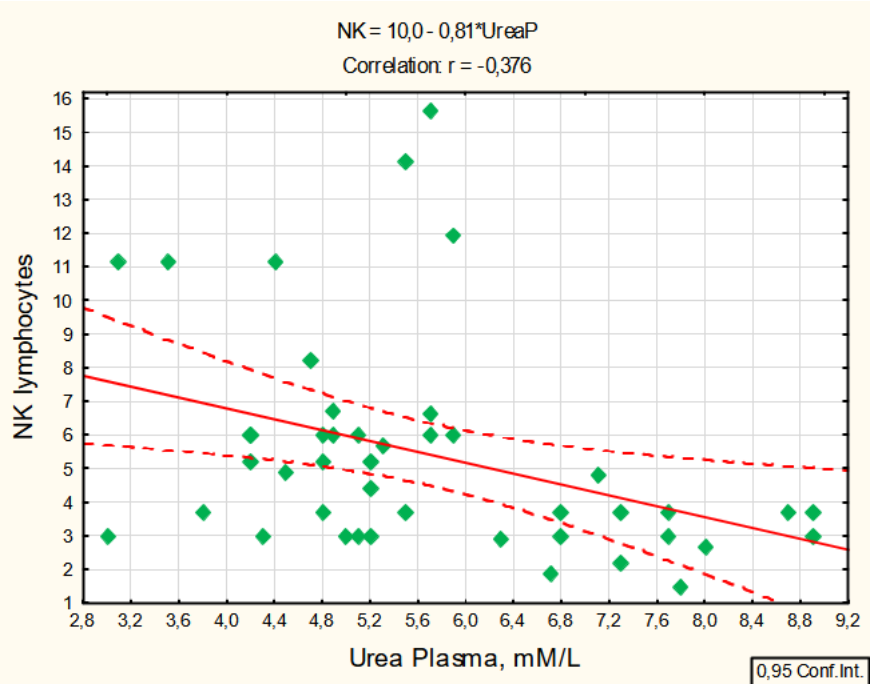


Fig. 8. Scatterplot of correlation between Urea Plasma level (X-line) and NK lymphocytes level (Y-line)

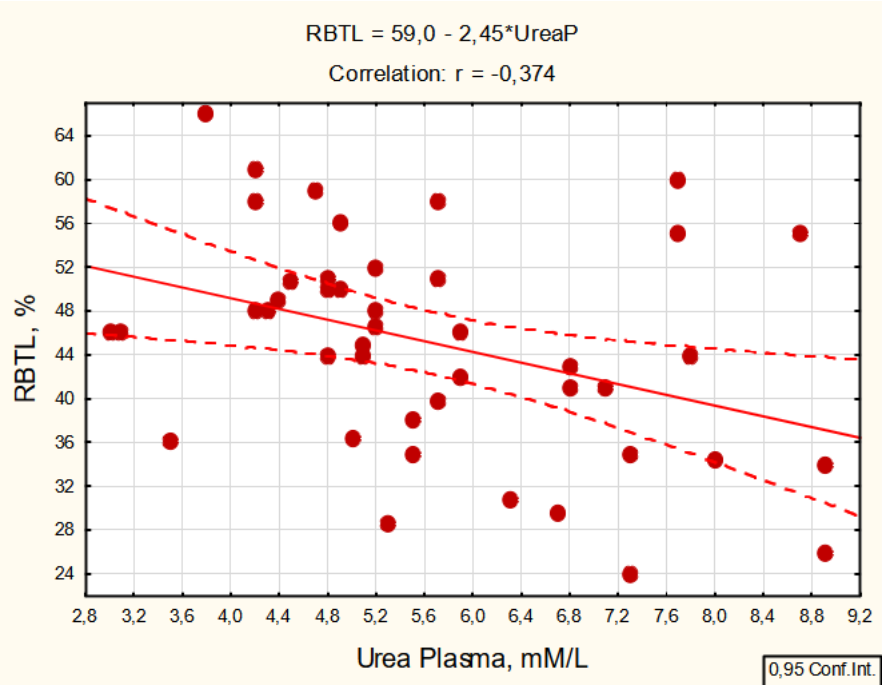
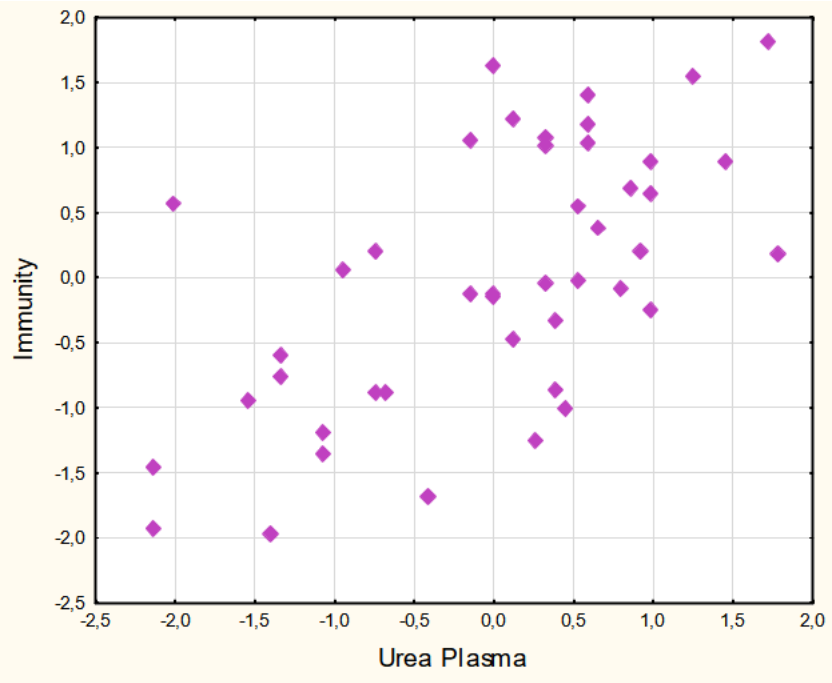


Fig. 8. Scatterplot of correlation between Urea Plasma level (X-line) and Blast transformation of T lymphocytes reaction (Y-line)



R=0,622; R^2=0,387; $\chi^2_{(4)}$=19,6; p<10^{-3}; Λ Prime=0,613

Fig. 9. Scatterplot of canonical correlation between Urea Plasma (X-line) and parameters of Immunity (Y-line)

The most numerous correlations were found for the concentration of Urea in the urine. In particular, it negatively correlates with serum CRP (Fig. 10), Complement and IgA levels, while positively correlates with the relative levels of Eosinophils, B-lymphocytes (Fig. 11) and theophylline-sensitive T-lymphocytes in the blood, as well as the absolute and relative levels of serum γ-globulines. Such constellation of parameters of Immunity is determined by Urea urine by 57% (Table 6 and Fig. 12).

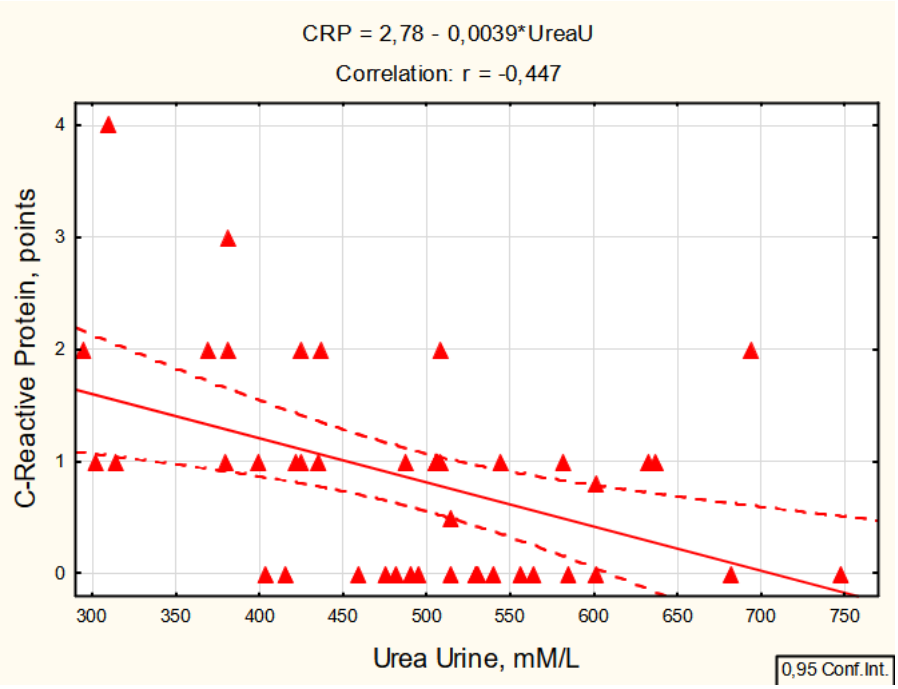


Fig. 10. Scatterplot of correlation between Urea Urine concentration (X-line) and C-reactive Protein level (Y-line)

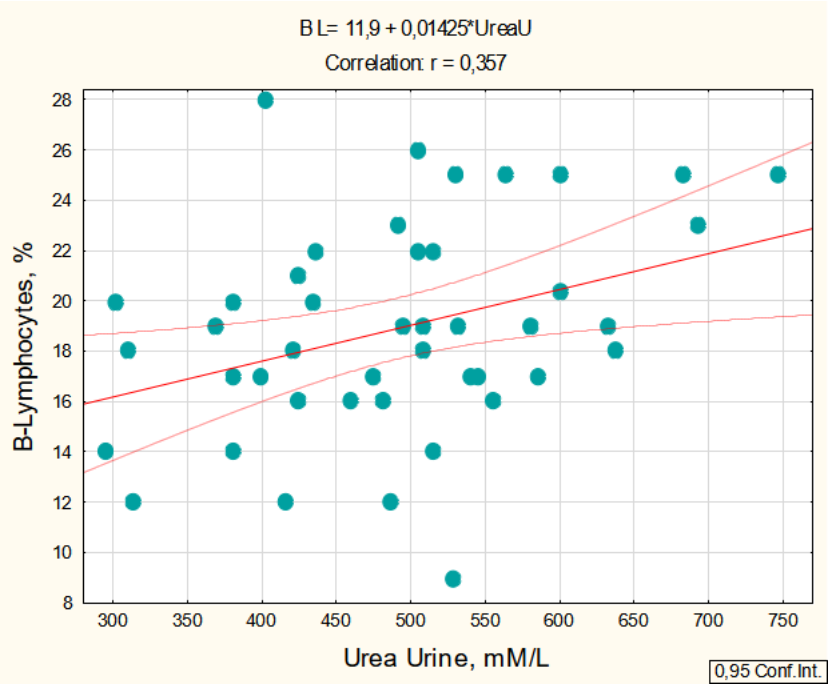
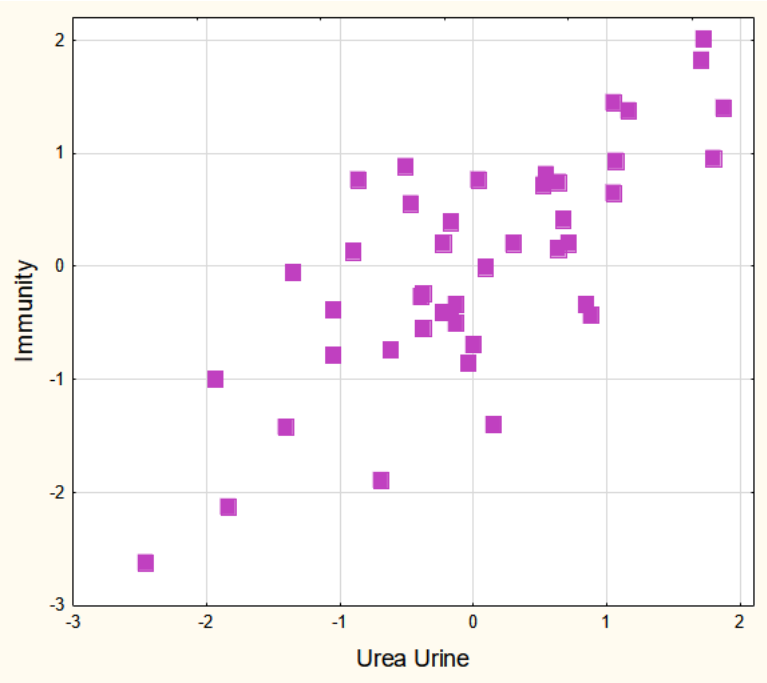


Fig. 11. Scatterplot of correlation between Urea Urine concentration (X-line) and B-Lymphocytes level (Y-line)

Table 6. Regression Summary for Urea Urine, mM/L
R=0,756; R^2=0,571; Adjusted R^2=0,473; $F_{(8,4)}$=5,8; p=0,0001

		Beta	St. Err. of Beta	B	SE of B	$t_{(35)}$	p-level
Variables	r		Intercpt	808	223	3,62	0,001
C-Reactive Protein, points	-0,45	-0,306	0,127	-34,77	14,37	-2,42	0,021
Complement, CH_{50}	-0,32	-0,243	0,119	-1,240	0,609	-2,04	0,049
IgA Serum, g/L	-0,28	-0,315	0,130	-249,0	102,9	-2,42	0,021
B Lymphocytes, %	0,36	0,299	0,137	7,494	3,429	2,19	0,036
Eosinophils, %	0,30	0,243	0,139	11,60	6,644	1,74	0,089
γ-globulines, g/L	0,27	0,697	0,404	17,97	10,42	1,72	0,093
γ-globulines, %	0,23	-0,668	0,399	-13,69	8,173	-1,68	0,103
Theophylline-sensitive T-Lymphocytes, %	0,23	0,232	0,131	5,616	3,177	1,77	0,086



R=0,756; R^2=0,571; $\chi^2_{(8)}$=32,2; p<10^{-4}; Λ Prime=0,429

Fig. 12. Scatterplot of canonical correlation between Urea Urine (X-line) and parameters of Immunity (Y-line)

In contrast, the immunotropic activity of Creatinine was the lowest. In particular, plasma Creatinine downregulates the level of natural killers (Fig. 13) and monocytes, as well as the mitogenic ability of T-lymphocytes, instead upregulates the level of lymphocytes in general. The degree of determination is 35% (Table 7, Fig. 14).

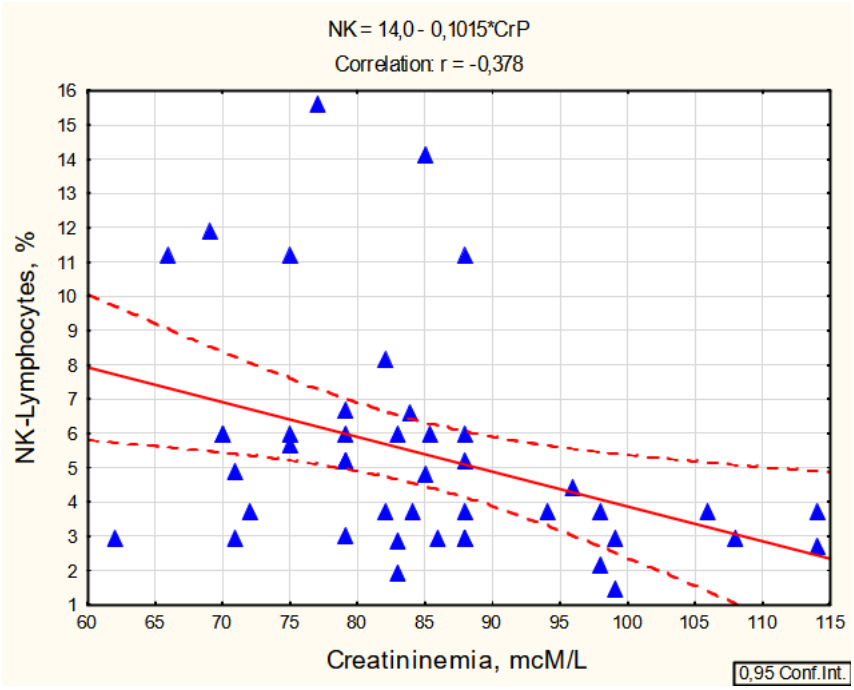
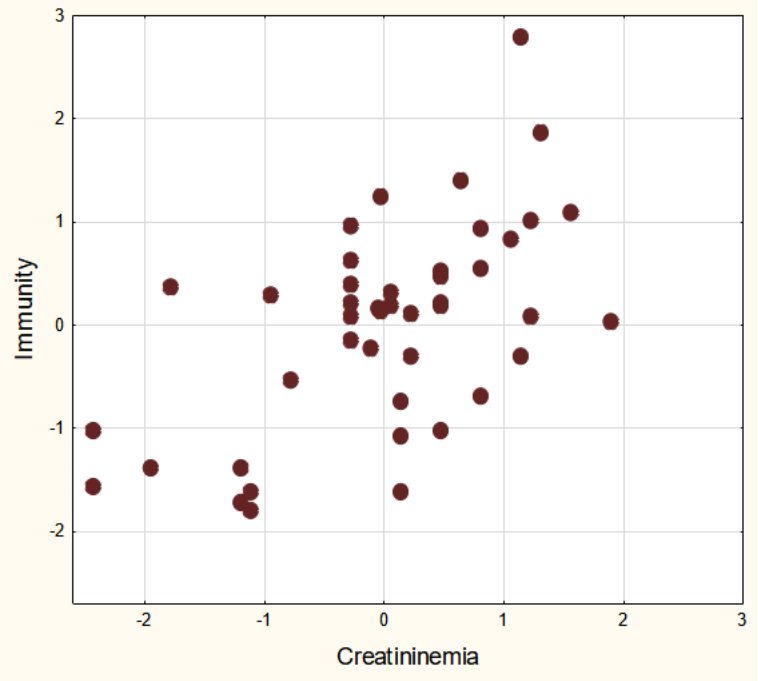


Fig. 13. Scatterplot of correlation between Creatininemia (X-line) and NK-Lymphocytes level (Y-line)

Table 7. Regression Summary for Creatininemia, µM/L
R=0,588; R^2=0,346; Adjusted R^2=0,278; $F_{(4,4)}$=5,1; p=0,002

		Beta	St. Err. of Beta	B	SE of B	$t_{(39)}$	p-level
Variables	r		Intercpt	96,75	9,516	10,2	10^{-6}
NK Lymphocytes, %	-0,38	-0,252	0,136	-0,938	0,506	-1,85	0,071
Monocytes, 10^9/L	-0,30	-0,309	0,130	-25,68	10,83	-2,37	0,023
Blast transformation of T-Lymphocytes, %	-0,29	-0,307	0,136	-0,375	0,166	-2,25	0,030
Pan-Lymphocytes, 10^9/L	0,25	0,284	0,137	6,068	2,925	2,07	0,045

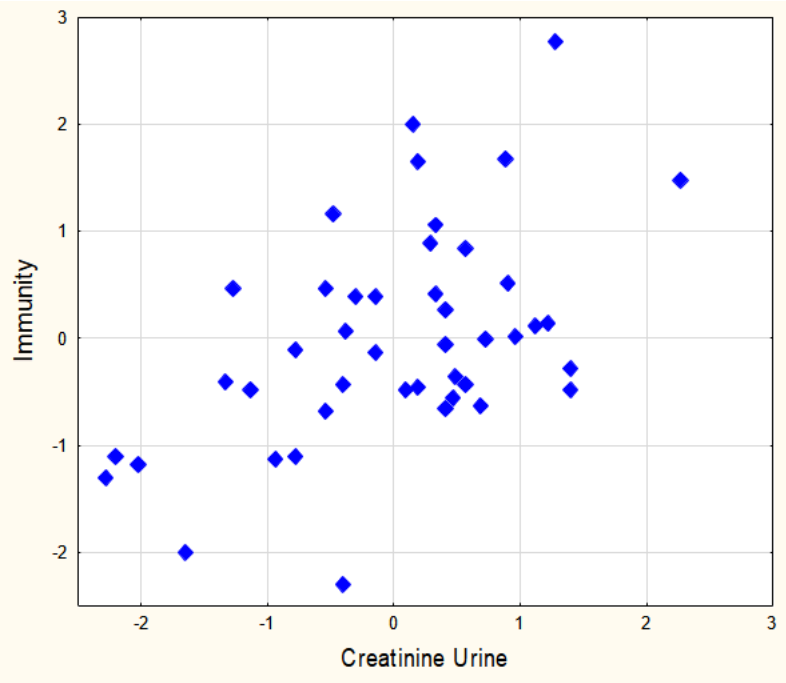


R=0,588; R^2=0,346; $\chi^2_{(4)}$=17,0; p=0,002; Λ Prime=0,654
Fig. 14. Scatterplot of canonical correlation between Creatininemia (X-line) and parameters of Immunity (Y-line)

The concentration of Creatinine in the urine is weakly negatively correlated with the activity and completeness of bacterial Phagocytosis by neutrophils and is also weakly positively correlated with the levels of γ-globulins, Natural Killers and Monocytes. Such constellation of parameters of Immunity is determined by Creatinine urine by 26% only (Table 8 and Fig. 15).

Table 8. Regression Summary for Creatinine Urine, mM/L
R=0,510; R^2=0,260; Adjusted R^2=0,163; $F_{(5,4)}$=2,7; p=0,036

		Beta	St. Err. of Beta	B	SE of B	$t_{(38)}$	p-level
Variables	r		Intercpt	6,468	1,407	4,60	10^{-4}
Phagocytose Index of Neutrophils, %	-0,29	-0,154	0,149	-0,020	0,019	-1,03	0,308
Killing Index of Neutrophils, %	-0,22	-0,257	0,150	-0,021	0,012	-1,71	0,095
γ-globulines, %	0,28	0,237	0,148	0,059	0,037	1,60	0,118
NK Lymphocytes, %	0,23	0,162	0,148	0,063	0,058	1,09	0,281
Monocytes, %	0,21	0,260	0,146	0,147	0,083	1,78	0,083



R=0,510; R^2=0,260; $\chi^2_{(5)}$=11,9; p=0,036; Λ Prime=0,740

Fig. 15. Scatterplot of canonical correlation between Creatinine Urine (X-line) and parameters of Immunity (Y-line)

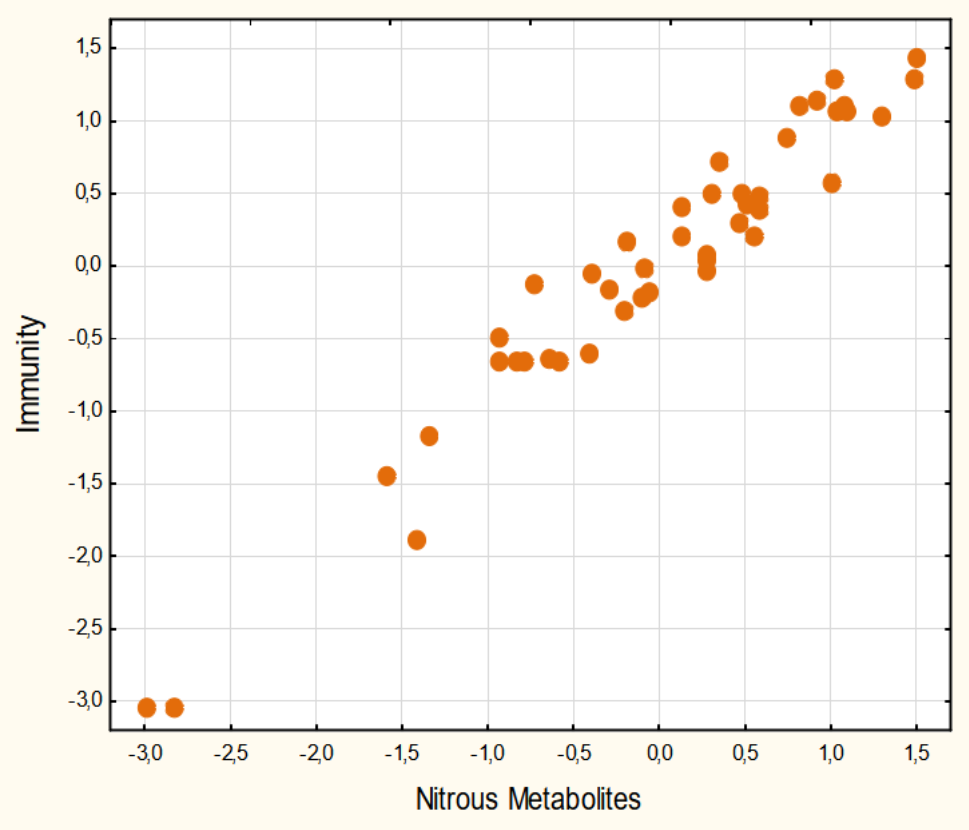
At the final stage, following the accepted algorithm, the canonical correlation between the parameters of nitrogen metabolism, on the one hand, and the parameters of immunity - on the other hand, is analyzed.

As a result of canonical analysis, two pairs of canonical roots were formed. Nitrogen root of the first pair (Table 9), judging by the factor loads, represents mainly Urea urine and plasma and Uric acid urine, and to a lesser extent Creatinine urine and plasma. Note the same sign of the factor loads. The immune root of the first pair contains information about the parameters of the **Phagocytosis**, **Cellular** and **Humoral** immunity as well as of the **Leukocytogram**, which are subject to **stimulating** or **suppressive** effects of Urine, Uric acid and Creatiniine. The immunomodulatory effect of these nitrogenous metabolites, judging by the coefficient of determination, is 94,3% (Fig. 16).

Table 9. Factor load on canonical roots of nitric metabolites and immunity parameters

Left set	**Root 1**	**Root 2**
Urea Urine	**-0,588**	**-0,386**
Uric acid Urine	**-0,568**	**-0,576**
Urea Plasma	**-0,505**	**0,299**
Creatinine Urine	**-0,305**	**0,267**
Creatininemia	**-0,199**	0,103
Bilirubinemia	-0,131	**0,409**
Uricemia	-0,053	**0,375**
Right set	**Root 1**	**Root 2**
B Lymphocytes, %	**-0,388**	**-0,387**
Bactericidal Capacity of Monocytes, 10^9 B/L	**-0,303**	-0,082
Phagocytosis Index of Monocytes, %	**-0,272**	0,140
γ-globulines, %	**-0,256**	0,059
γ-globulines, g/L	**-0,248**	-0,077
Pan-Lymphocytes, 10^9/L	**-0,244**	0,005
Monocytes, 10^9/L	**-0,192**	0,033

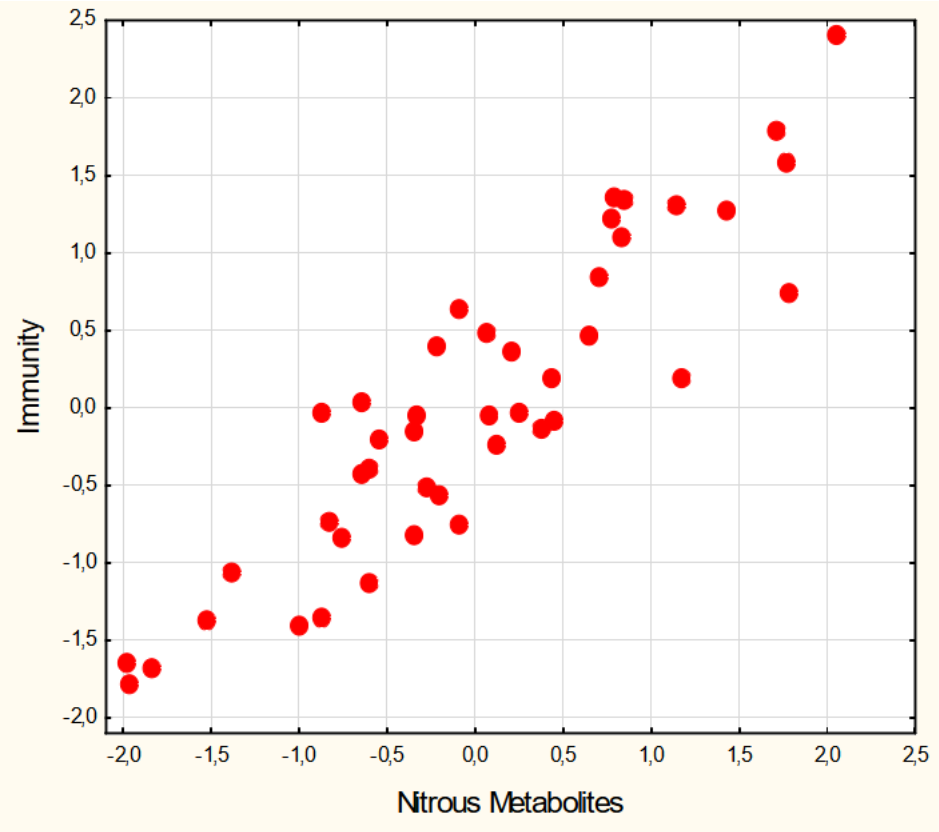
Monocytes, %	**-0,156**	0,015
IgA Serum, g/L	**0,447**	**-0,160**
Complement, CH_{50}	**0,252**	**0,240**
C-Reactive Protein, points	**0,190**	**0,175**
NK Lymphocytes, %	**0,084**	-0,036
IgM Serum, g/L	-0,044	**0,375**
Polymorphonucleary Neutrophils, %	0,105	**0,257**
Theophylline-sensitive T-Lymphocytes, %	**-0,175**	**0,240**
Phagocytose Index of Neutrophils, %	-0,060	**0,081**
Eosinophils, %	-0,134	**-0,347**
Lysozyme, nM/L	0,051	**-0,389**
Microbial Count of Neutrophils, Bac/Phag	0,077	**-0,241**
Blast transformation of T-Lymphocytes, %	0,054	**-0,235**
Killing Index of Neutrophils, %	-0,080	**-0,155**
Theophylline-resistant T-Lymphocytes, %	-0,001	**-0,028**



R=0,971; R^2=0,943; $\chi^2_{(15)}$=239; $p<10^{-5}$; Λ Prime=0,0002

Fig. 16. Scatterplot of canonical correlation between Nitrous Metabolites (X-line) and the Immunity (Y-line) in humans. First pair of Roots

The nitrogen root of the second pair receives a similar in magnitude and sign factor load from Uric acid of urine and less, but also a negative load from Urea of urine, instead of opposite in sign load from Urea of plasma and both Creatinine parameters. The biggest difference in the factor structure is the significant load from Bilirubinemia and Uricemia. Their immunomodulatory effect is directed as a rule to another constellation of immune parameters and is much weaker (Fig. 17). Attention should be paid to identical factor loads on B-lymphocytes, Complement and C-reactive protein.



R=0,904; R^2=0,817; $\chi^2_{(12)}$=158; p=0,028; Λ Prime=0,004

Fig. 17. Scatterplot of canonical correlation between Nitrous Metabolites (X-line) and the Immunity (Y-line) in humans. Second pair of Roots

CONCLUSION

The results of clinical and physiological observation, in principle, confirm our previous experimental data [32] on the significant immunotropic activity of nitrogenous metabolites. As we have already noted, the immunomodulatory effect of bilirubin is probably mediated through aryl hydrocarbon receptors [2,6,7,9,29,33,37], and uric acid through TL- and Adenosine receptors [1,5,10,11,19,20,25-27,36] of immune cells. The question of mediators of the immunomodulatory action of urea and creatinine remains open and will be the subject of the next article. And now let's limit ourselves to the announcement of the mediating role of mediators of the autonomic nervous system and adaptation hormones in line with the concepts of neuroendocrine-immune complex and functional-metabolic continuum [13,18].

ACKNOWLEDGMENT

We express sincere gratitude to administration of sanatorium “Perlyna Prykarpattya” of Ministry of Internal Affairs of Ukraine for help in conducting this investigation.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975 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. Apasov S, Chen JF, Smith P, Sitkovsky M. A_{2A} receptor dependent and A_{2A} receptor independent effects of extracellular adenosine on murine thymocytes in condicion of adenosine deaminase deficiency. Blood. 2000; 95(12): 3859-3867.
2. Avilla MN, Malecki KMC, Hahn ME, Wilson RH, Bradfield CA. The Ah Receptor: Adaptive Metabolism, Ligand Diversity, and the Xenokine Model. Chem Res Toxicol. 2020; 33(4): 860-879.
3. Bianco C. Population of lymphocytes bearing a membrane receptor for antigen-antibody complex. J Exp Med. 1970; 134(4): 702-720.
4. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(1): 68-102.
5. Carvalho LAC, Lopes JPPB, Kaihami GH, Silva RP, Bruni-Cordoso A, Baldini RL, Meotti FC. Uric acid disrupts hypochlorous acid production and bactericidal activity of HL-60 cells. Redox Biology. 2018; 16: 179-188.
6. Climaco-Arvizu S, Domínguez-Acosta O, Cabañas-Cortés MA, et al. Aryl hydrocarbon receptor influences nitric oxide and arginine production and alters M1/M2 macrophage polarization. Life Sci. 2016; 155: 76-84.
7. Díaz-Díaz CJ, Ronnekleiv-Kelly SM, Nukaya M, et al. The Aryl Hydrocarbon Receptor is a Repressor of Inflammation-associated Colorectal Tumorigenesis in Mouse. Ann Surg. 2016; 264(3): 429-436.
8. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil; 1981: 110 p.
9. Esser C, Rannug A. The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. Pharmacol Rev. 2015; 67(2): 259-279.
10. Gao L, Jiang Y, Wang Y, Qu X, Li L, Lou X, Wang Y, GuoW, Liu Y. Male asymptomatic hyperuricemia patients display a lower number of $NKG2D^+$ NK cells before and after a low-purine diet. Medicine (Baltimore). 2018; 97(50): e13668.
11. Ghaemi-Oskouie F, Shi Yan. The role of uric acid as an endogenous danger signal in immunity and inflammation. Curr Rheumatol Rep. 2011; 13(2): 160-166.
12. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa: Astroprint; 1998: 608 p.
13. Gozhenko AI. Functional-metabolic continuum [in Russian]. J of NAMS of Ukraine. 2016; 22 (1): 3-8.
14. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Popovych IL. Functional relationships between parameters of uric acid exchange and immunity in female rats. Actual problems of transport medicine. 2019; 4(58): 123–131.
15. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Popovych IL. Features of immune status in different states of uric acid metabolism in female rats. Journal of Education, Health and Sport. 2019; 9(12): 167-180.
16. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Kovbasnyuk MM, Popovych IL. Relationships between parameters of uric acid exchange and immunity as well as microbiota in patients with neuroendocrine-immune complex dysfunction. Journal of Education, Health and Sport. 2020; 10(1): 165-175.
17. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Kovbasnyuk MM, Popovych IL. Relationships between changes in uric acid parameters metabolism and parameters of immunity and microbiota in patients with neuroendocrine-immune complex dysfunction. Journal of Education, Health and Sport. 2020; 10(2): 212-222.
18. Gozhenko AI, Zukow W, Polovynko IS, Zajats LM, Yanchij RI, Portnichenko VI, Popovych IL. Individual Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment. RSW. UMK. Radom. Torun; 2019: 200 p.
19. Hoskin DW, Mader JS, Furlong SJ, Conrad DM, Blay J. Inhibition of T cell and NK cell function by adenosine and its contribution to immune evasion by tumor cells (Review). Int J Oncol. 2008; 32(3): 527-535.
20. Huang S, Apasov S, Koshiba M, SitkovskiM. Role of A_{2A} extracellular adenosine receptor mediated signaling in adenosine mediated inhibition of of T-cell activation and expansion.

Blood. 1997; 90(4): 1600-1610.
21. Jondal M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J Exp Med. 1972; 136(2): 207-215.
22. Kostyuk PG, Popovych IL, Ivassivka SV (editors). Chornobyl', Adaptive and Defensive Systems, Rehabilitation [in Ukrainian]. Kyiv. Computerpress; 2006: 348 p.
23. Limatibul S, Shore A, Dosch HM, Gelfand EW. Theophylline modulation of E-rosette formation: an indicator of T-cell maturation. Clin Exp Immunol. 1978; 33(3): 503-513.
24. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry. 1965; 2(3): 235-254.
25. Martínez-Reyes CP, Manjarrez-Reyna AN, Méndez-García LA, et al. Uric Acid Has Direct Proinflammatory Effects on Human Macrophages by Increasing Proinflammatory Mediators and Bacterial Phagocytosis Probably via URAT1. Biomolecules. 2020; 10(4): 576.
26. Morelli M, Carta AR, Kachroo A, Schwarzschild A. Pathophysiological roles for purines: adenosine, caffeine and urate. Prog Brain Res. 2010; 183: 183-208.
27. Navalta JW, Fedor EA, Schafer MA, Lyons TS, Tibana RA, Pereira GB, Prestes J. Caffeine affects $CD8^+$ lymphocyte differently in naïve and familiar individuals following moderate intensity exercise. Int J Immunopathol Pharmacol. 2016; 29(2): 288-294.
28. Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM. Immune status, principles of its evaluation and correction of immune disorders [in Russian]. Kyiv. Zdorovya; 1995: 211 p.
29. Phelan D, Winter GM, Rogers WJ, Lam JC, Denison MS. Activation of the Ah receptor signal transduction pathway by bilirubin and biliverdin. Arch Biochem Biophys. 1998; 357(1): 155-163.
30. Popadynets' OO, Gozhenko AI, Zukow W, Popovych IL. Relationships between the entropies of EEG, HRV, immunocytogram and leukocytogram. Journal of Education, Health and Sport. 2019; 9(5): 651-666.
31. Popovych IL, Flyunt IS, Alyeksyeyev OI, Barylyak LG, Bilas VR. Sanogenetic Bases of Rehabilitation on Spa Truskavets' Urological Patients from Chornobylian Contingent [in Ukrainian]. Kyiv. Computerpress; 2003: 192 p.
32. Popovych IL, Gozhenko AI, Kuchma IL, Zukow W, Bilas VR, Kovalchuk GY, Ivasivka AS. Immunotropic effects of so-called slag metabolites (creatinine, urea, uric acid and bilirubin) at rats. Journal of Education, Health and Sport. 2020; 10(11): 320-336.
33. Quintana FJ, Sherr DH. Aryl hydrocarbon receptor control of adaptive immunity. Pharmacol Rev. 2013; 65(4): 1148-1161.
34. Romodanov AP (editor). Postradiation Encephalopathy. Experimental Researches and Clinical Observations [in Ukrainian and Russian]. Kyiv. USRI of Neurosurgery; 1993: 224 p.
35. Smagliy VS, Gozhenko AI, Korda IV, Badiuk NS, Zukow W, Kovbasnyuk MM, Popovych IL. Variants of uric acid metabolism and their immune and microbiota accompaniments in patients with neuroendocrine-immune complex dysfunction. Actual problems of transport medicine. 2020; 1(59): 114–125.
36. Vigano S, Alatzoglou D, Irving M, Menetrier-Caux Ch, Caux Ch, Romero P, Coukos G. Targeting adenosine in cancer immunotherapy to enhance T-cell function. Front Immunol. 2019; 10: 925.
37. Yang X, Liu H, Ye T, et al. AhR activation attenuates calcium oxalate nephrocalcinosis by diminishing M1 macrophage polarization and promoting M2 macrophage polarization. Theranostics. 2020; 10(26): 12011-12025.