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IMMUNOTROPIC EFFECTS OF NITROGENOUS METABOLITES IN HEALTHY HUMANS

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Abstract

Background. We have previously shown that nitrogenous metabolites have immunomodulatory effects, both in healthy rats and in humans exposed to pathogenic influences. The purpose of this study is their immunotropic activity in clinically healthy people. **Materials and Methods**. The object of observation were 27 men (aged 24-63 ys) and 14 women (33-62 ys). The plasma levels of the nitrogenous metabolites and parameters of immunity twice with an interval of 5 days was performed. **Results**. Judging by the multiple correlation coefficient uric acid exhibits maximal immunotropic activity (R=0,665), followed by creatinine (R=0,596) and urea (R=0,541), and closes the constellation of metabolites bilirubin, with the activity of conjugated bilirubin predominating over that of unconjugated (0,539 vs 0,484). Nitrogenous metabolites together upregulate the level in the blood of B-lymphocytes, CIC, IgG, IL-1, eosinophils and monocytes, as well as most parameters of phagocytosis by neutrophils Staph. aureus and E. coli. Instead, they downregulate the phagocytosis activity of Staph. aureus, the relative content of rod-shaped neutrophils, lymphocytes in general and NK-, T active and 0-Lymphocytes in particular. Downregulation of 0-Lymphocytes reflects upregulation of receptor expression, apparently CD22. **Conclusion**. Nitrogenous metabolites exhibit immunotropic activity in both healthy rats and humans, as well as in patients.

Key words: **urea, uric acid, creatinine, bilirubin, immunity, relationships, humans.**

INTRODUCTION

We have previously shown that nitrogenous metabolites have immunomodulatory effects, both in healthy rats [9,10,28] and in humans exposed to pathogenic influences [11,12,16,31]. With regard to uric acid, it is known that the magnitude and even the sign of the correlation coefficients with the parameters of the human body depend on the state of adaptation, the presence or absence of chronic pyelonephritis, as well as its phase [14]. Therefore, the aim of this study is immunotropic activity of nitrogenous metabolites in clinically healthy people.

MATERIAL AND METHODS

The object of observation were 27 men (aged 24-63 ys) and 14 women (33-62 ys). The survey was performed twice with an interval of 5 days.

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 analyzes were carried out according to the instructions described in the manual [7]. The analyzers “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) 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 [19,22]. 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 (T-active) determined by test of “active” rosette formation. The state of humoral immunity judged by the concentration in serum of Circulating Immune Complexes (by polyethylene glycol precipitation method) and Immunoglobulins classes M, G, A (ELISA, analyser “Immunochem”, USA). In addition, the level of IL-1 was determined (by the ELISA with the use of analyzer “RT-2100C” and corresponding set of reagents from “Diactone”, France).

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [3] with moderately modification by MM Kovbasnyuk [12,24]. 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 [25,27]:

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

In portion of capillary blood we counted up Leukocytogram (LCG) (Eosinophils, Rod-shaped and Polymorphonucleary Neutrophils, Lymphocytes and Monocytes).

We calculated also the Entropy (h) of Immunocytogram (ICG) and Leukocytogram (LCG) using IL Popovych’s formulas [24,26] derived from classical CE Shannon’s formula [30]:

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

$$hLCG = - [L \bullet \log_2 L + M \bullet \log_2 M + E \bullet \log_2 E + PMNN \bullet \log_2 PMNN + RSN \bullet \log_2 RSN]/\log_2 5$$

Results processed by using the software package "Statistica 64".

RESULTS AND DISCUSSION

Following the accepted algorithm, we first created a matrix of correlations between nitrogenous metabolites and immune parameters (Table 1).

Table 1. Matrix of correlations between nitrogenous metabolites and immunity parameters in healthy men

	Correlations (n=82)				
Variable	BilNC	BilC	Creat	Urea	UA
Bilirub Unconjugated	1,00	0,78	0,18	0,30	0,02
Bilirubine Conjugated	0,78	1,00	0,25	0,26	0,26
Creatinine	0,18	0,25	1,00	0,33	0,56
Urea	0,30	0,26	0,33	1,00	0,06
Uric acid	0,02	0,26	0,56	0,06	1,00
IL-1	0,15	0,11	0,14	0,13	0,09
CD4	-0,12	-0,14	0,07	-0,00	0,04
CD8	-0,10	0,03	0,06	-0,12	0,09
T-active	-0,07	-0,20	0,13	-0,17	0,18
CD22	0,06	0,27	0,43	0,05	0,45
CD56	0,13	0,04	-0,09	0,08	-0,11
0-Lymph	-0,00	-0,21	-0,42	-0,02	-0,43
Entropy ICG	0,11	0,21	0,26	-0,04	0,27
CIC	-0,02	0,18	0,33	0,00	0,31
IgG	-0,11	-0,06	-0,00	0,08	0,13
IgA	0,05	0,02	0,26	0,09	0,24
IgM	0,01	0,08	-0,01	0,04	0,11
Phl vs St. aur.	-0,07	-0,06	-0,18	-0,31	0,01
MC for St. aur.	-0,08	0,12	-0,16	-0,16	0,08
Killing St. aur.	-0,18	-0,09	0,12	0,08	0,19
Bactericidity vs St.aur.	-0,24	-0,05	0,15	0,09	0,31
Phl vs E. coli	-0,31	-0,25	0,08	-0,27	0,28
MC for E. coli	-0,18	-0,05	-0,05	-0,05	0,05
Killing E. coli	-0,13	0,11	0,05	0,08	0,15
Bactericidity vs E. coli	-0,26	-0,02	0,11	0,12	0,26
Leukocytes	-0,15	-0,13	0,20	0,12	0,22
Eosinophils	0,23	0,22	0,08	0,17	0,11
Stub Neutrophils	-0,04	-0,06	-0,12	-0,11	-0,15
Polymorphonucl Neutr	-0,12	0,00	0,05	0,02	0,18
Pan-Lymphocytes	0,02	-0,07	-0,18	-0,02	-0,34
Monocytes	0,06	-0,03	0,25	-0,02	0,22

In the next step of the analysis, a regression model was constructed for each nitrogenous metabolite by stepwise exclusion until the maximum level of adjusted R^2 was reached.

As expected, based on the previous results, uric acid showed the maximum immunotropic activity among nitrogenous metabolites (Table 2).

Table 2. Regression Summary for Uricemia, µM/L
R=0,665; R^2=0,442; Adjusted R^2=0,345; $F_{(12,7)}$=4,55; $p<10^{-4}$

		Beta	St. Err. of Beta	B	SE of B	$t_{(69)}$	p-level
Variables	r		Intercpt	-3059	1094	-2,80	0,007
$CD22^+$ B Lymphocytes, %	**0,45**	0,668	0,265	8,513	3,383	2,52	0,014
CIC Serum, units	**0,31**	0,132	0,119	0,584	0,529	1,10	0,273
Bactericidal Capacity vs St. aur, 10^9 B/L	**0,31**	-0,498	0,238	-1,155	0,551	-2,10	0,040
Phagocytose Index vs E. coli, %	**0,28**	0,279	0,106	28,10	10,73	2,62	0,011
Leukocytes, 10^9/L	**0,22**	0,327	0,159	21,31	10,35	2,06	0,043
Killing Index vs Staph. aureus, %	**0,19**	0,317	0,164	2,453	1,268	1,94	0,057
Active T-Lymphocytes, %	**0,18**	0,205	0,110	3,633	1,948	1,86	0,066
Killing Index vs E. coli, %	**0,15**	0,268	0,131	2,639	1,290	2,05	0,045
IgG Serum, g/L	**0,13**	0,158	0,117	3,495	2,586	1,35	0,181
Eosinophils, %	**0,11**	0,136	0,101	5,994	4,464	1,34	0,184
0 Lymphocytes, %	**-0,43**	0,355	0,273	3,932	3,028	1,30	0,198
Pan-Lymphocytes, 10^9/L	**-0,34**	-0,236	0,116	-3,319	1,631	-2,03	0,046

Uric acid upregulates parameters of humoral and cellular immunity, as well as phagocytosis of gram-positive and gram-negative bacteria by neutrophils.

The negative correlation with the levels of 0- and pan-lymphocytes reflects, in our opinion, the upregulation of the expression of immature lymphocytes primarily CD22 receptors and/or the prevention of loss of these receptors. The degree of positive determination of uric acid immune status is 44%.

Creatinine, like uric acid, upregulates humoral and cellular immunity, while downgulates the activity and intensity of phagocytosis of gram-positive bacteria (Table 3).

Table 3. Regression Summary for Creatinine, µM/L
R=0,596; R^2=0,356; Adjusted R^2=0,295; $F_{(7,7)}$=5,83; $p<10^{-4}$

		Beta	St. Err. of Beta	B	SE of B	$t_{(74)}$	p-level
Variables	r		Intercpt	271	200	1,36	0,179
$CD22^+$ B Lymphocytes, %	**0,43**	0,380	0,100	0,820	0,216	3,79	0,0003
CIC Serum, units	**0,33**	0,218	0,107	0,163	0,080	2,04	0,045
Monocytes, %	**0,25**	0,107	0,101	0,588	0,551	1,07	0,290
Leukocytes, 10^9/L	**0,20**	0,097	0,097	1,071	1,069	1,00	0,320
Active T-Lymphocytes, %	**0,13**	0,147	0,098	0,440	0,294	1,50	0,138
Phagocytose Index vs Staph. aureus, %	**-0,18**	-0,105	0,101	-2,105	2,040	-1,03	0,305
Microbial Count for St. aur., Bac/Phag	**-0,16**	-0,216	0,105	-0,306	0,148	-2,07	0,042

Urea downregulates the activity of phagocytosis by neutrophils of both types of bacteria and levels in the blood of T-killers and rod-shaped neutrophils, instead upregulates the level of eosinophils (Table 4).

Table 4. Regression Summary for Urea Plasma, mM/L
R=0,541; R^2=0,292; Adjusted R^2=0,236; $F_{(6,8)}$=5,17; $p<10^{-3}$

		Beta	St. Err. of Beta	B	SE of B	$t_{(75)}$	p-level
Variables	r		Intercpt	84,9	18,2	4,68	$<10^{-4}$
Phagocytose Index vs Staph. aureus, %	**-0,31**	-0,330	0,104	-0,478	0,150	-3,18	0,002
Phagocytose Index vs E. coli, %	**-0,27**	-0,255	0,102	-0,313	0,126	-2,49	0,015
$CD8^+$ T-cytolytic Lymphocytes, %	**-0,12**	-0,338	0,108	-0,053	0,017	-3,14	0,002
Rod-shaped Neutrophils, %	**-0,11**	-0,167	0,100	-0,129	0,077	-1,68	0,097
Eosinophils, %	**0,17**	0,198	0,102	0,106	0,055	1,93	0,058
Bactericidal Capacity vs E. coli, 10^9 B/L	**0,12**	0,225	0,104	0,007	0,003	2,17	0,033

Interestingly, the profiles of the immunotropic effects of conjugated and unconjugated bilirubin have both common and significantly different features (Tables 5 and 6). In particular, both forms of bilirubin upregulates the levels of eosinophils and IL-1 as well as downregulates the levels of T-helpers and the activity of phagocytosis of E. coli. However, bilirubin conjugated upregulates the levels of B-lymphocytes and the intensity of phagocytosis of Staph. aureus as well as complete of phagocytosis of E. coli while downregulates the levels of T active and 0-lymphocytes, whereas bilirubin unconjugated with respect to these parameters is ineffective. On the other hand, bilirubin unconjugated downregulates the levels of the four phagocytosis parameters and upregulates the level of NK-lymphocytes, whereas these parameters are not affected by conjugated bilirubin.

Table 5. Regression Summary for Bilirubin conjugated, µM/L
R=0,539; R^2=0,291; Adjusted R^2=0,202; $F_{(9,7)}$=3,28; p=0,002

		Beta	St. Err. of Beta	B	SE of B	$t_{(72)}$	p-level
Variables	r		Intercpt	262	67	3,40	0,001
$CD22^+$ B Lymphocytes, %	**0,27**	-3,213	1,703	-2,102	1,114	-1,89	0,063
Eosinophils, %	**0,22**	0,143	0,104	0,324	0,235	1,38	0,172
Microbial Count for St. aur., Bac/Phag	**0,12**	0,244	0,124	0,105	0,053	1,97	0,053
Killing Index vs E. coli, %	**0,11**	-0,225	0,140	-0,114	0,071	-1,61	0,111
Interleukin-1, ng/L	**0,11**	0,642	0,312	1,825	0,886	2,06	0,043
Phagocytose Index vs E. coli, %	**-0,25**	-0,343	0,119	-1,773	0,617	-2,87	0,005
0 Lymphocytes, %	**-0,21**	-3,712	1,796	-2,111	1,021	-2,07	0,042
Active T-Lymphocytes, %	**-0,20**	-0,163	0,110	-0,149	0,100	-1,49	0,141
$CD4^+$ T-helper Lymphocytes, %	**-0,14**	-1,182	0,534	-0,878	0,397	-2,21	0,030

Table 6. Regression Summary for Bilirubin unconjugated, µM/L
R=0,484; R^2=0,234; Adjusted R^2=0,138; $F_{(9,7)}$=2,44; p=0,017

		Beta	St. Err. of Beta	B	SE of B	$t_{(72)}$	p-level
Variables	r		Intercpt	53,5	17,1	3,13	0,003
Phagocytose Index vs E. coli, %	**-0,31**	-0,366	0,125	-0,499	0,170	-2,93	0,005
Bactericidal Capacity vs E. coli, 10^9 B/L	**-0,26**	-0,525	0,270	-0,019	0,010	-1,94	0,056
Bactericidal Capacity vs St. aur, 10^9 B/L	**-0,24**	0,389	0,288	0,012	0,009	1,35	0,181
Killing Index vs Staph. aureus, %	**-0,18**	-0,321	0,180	-0,034	0,019	-1,78	0,079
Microbial Count for E. coli, Bac/Phag	**-0,18**	0,235	0,168	0,022	0,016	1,40	0,165
$CD4^+$ T-helper Lymphocytes, %	**-0,12**	-0,233	0,191	-0,045	0,037	-1,21	0,229
Eosinophils, %	**0,23**	0,168	0,109	0,100	0,065	1,54	0,127
Interleukin-1, ng/L	**0,15**	0,359	0,204	0,268	0,152	1,76	0,082
$CD56^+$ NK Lymphocytes, %	**0,13**	-0,396	0,289	-0,064	0,047	-1,37	0,175

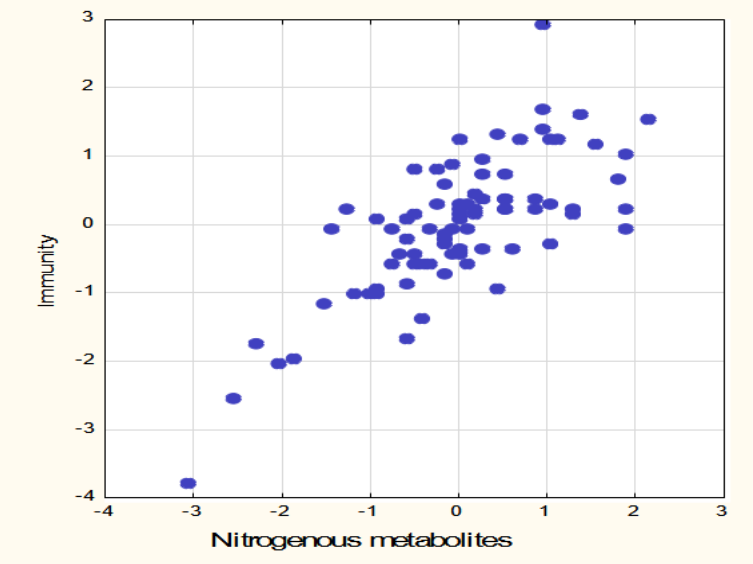
At the final stage, the canonical correlation between nitrogenous metabolites, on the one hand, and immunity parameters, on the other, was analyzed. According to the results of the analysis, two pairs of canonical roots were formed.

The nitrogenous root of the first pair receives from uric acid and creatinine positive factor loadings, and from urea and bilirubin - negative. The immune canonical root is represented by 10 parameters subject to **upregulation** and three parameters subject to **downregulation** by uric acid and creatinine, as well as the level of eosinophils subject to **upregulation** by urea and bilirubin (table 7).

The total immunomodulatory effect of nitrogenous metabolites, judging by the coefficient of determination, is 55% (Fig. 1).

Table 7. Factor load on first pair of canonical roots of nitrogenous metabolites and immunity parameters

Left set	**Root 1**
Uric acid	**0,519**
Creatinine	**0,321**
Urea	**-0,572**
Bilirubin conjugated	**-0,488**
Bilirubin unconjugated	**-0,449**
Right set	**Root 1**
Phagocytose Index vs E. coli, %	**0,622**
Active T-Lymphocytes, %	**0,511**
Monocytes, %	**0,350**
Leukocytes, 10^9/L	**0,242**
$CD22^+$ B Lymphocytes, %	**0,239**
Phagocytose Index vs Staph. aureus, %	**0,209**
Bactericidal Capacity vs Staph. aur, 10^9 B/L	**0,200**
CIC Serum, units	**0,192**
$CD4^+$ T-Lymphocytes, %	**0,172**
Killing Index vs Staph. aureus, %	**0,162**
0-Lymphocytes, %	**-0,280**
Pan-Lymphocytes, 10^9/L	**-0,236**
$CD56^+$ NK Lymphocytes, %	**-0,183**
Eosinophils, %	**-0,161**



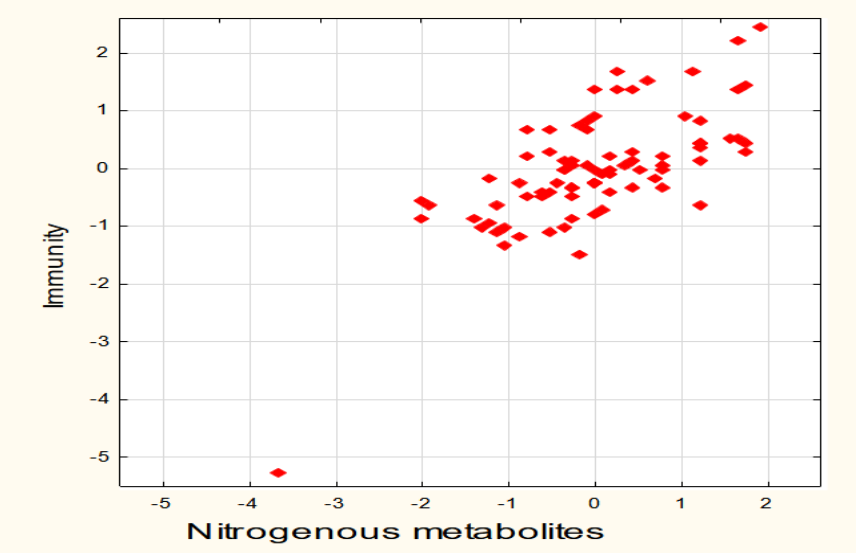
R=0,743; R^2=0,552; $\chi^2_{(105)}$=172; p<10^{-4}; Λ Prime=0,078

Fig. 1. Scatterplot of canonical correlation between Nitrogenous Metabolites (X-line) and the Immunity (Y-line) in healthy humans. First pair of Roots

The nitrogenous root of the second pair receives unidirectional factor loads from its elements, so the interpretation of **upregulating** and **downregulating** effects seems unambiguous (Table 8 and Fig. 2).

Table 8. Factor load on second pair of canonical roots of nitrogenous metabolites and immunity parameters

Left set	**Root 2**
Uric acid	**-0,752**
Creatinine	**-0,693**
Urea	**-0,529**
Bilirubin conjugated	**-0,473**
Bilirubin unconjugated	-0,077
Right set	**Root 2**
$CD22^+$ B Lymphocytes, %	**-0,669**
CIC Serum, units	**-0,504**
Bactericidal Capacity vs E. coli, 10^9 B/L	**-0,483**
Bactericidal Capacity vs Staph. aur, 10^9 B/L	**-0,458**
Killing Index vs E. coli, %	**-0,385**
Leukocytes, 10^9/L	**-0,292**
Killing Index vs Staph. aureus, %	**-0,283**
Eosinophils, %	**-0,202**
IgG Serum, g/L	**-0,168**
Interleukin-1, ng/L	**-0,165**
Monocytes, %	**-0,121**
Microbial Count for E. coli, Bac/Phag	-0,087
Microbial Count for Staph. aureus, Bac/Phag	-0,077
Phagocytose Index vs E. coli, %	-0,056
$CD4^+$ T-Lymphocytes, %	-0,015
0-Lymphocytes, %	**0,626**
Pan-Lymphocytes, 10^9/L	**0,366**
Phagocytose Index vs Staph. aureus, %	**0,250**
Rod-shaped Neutrophils, %	**0,223**
$CD56^+$ NK Lymphocytes, %	0,131
Active T-Lymphocytes, %	0,079



R=0,704; R^2=0,495; $\chi^2_{(80)}$=118; p=0,004; Λ Prime=0,175

Fig. 2. Scatterplot of canonical correlation between Nitrogenous Metabolites (X-line) and the Immunity (Y-line) in healthy humans. Second pair of Roots

Thus, nitrogenous metabolites together upregulates the levels in the blood of B-lymphocytes, CIC, IgG, IL-1, eosinophils and monocytes, as well as most parameters of phagocytosis by neutrophils of Staphylococcus aureus and E. coli. Instead, they downregulates the phagocytosis activity of Staphylococcus aureus, the relative content of rod-shaped neutrophils, lymphocytes in general and NK-, T active and 0-lymphocytes in particular. Downregulation of 0-lymphocytes reflects the upregulation of the expression of immature lymphocytes primarily CD22 receptors and/or the prevention of loss of these receptors.

Maximal immunotropic activity exhibits uric acid. This is due to its ability to affect immunocytes both directly, through TL-receptors [5,6,20] and adenosine receptors [21], and indirectly, through modulation of the immunotropic effects of the autonomic and endocrine systems [15,17,18] as well as lipids peroxidation [8,17]. The immunotropic activity of bilirubin is realized both in the latter way and through aryl hydrocarbon receptors [1,2,4,23,29,32].

For creatinine and urea specific receptors are unknown to us, therefore their immunotropic effects revealed by us are realized, probably, exclusively through the autonomic and endocrine systems and lipids peroxidation.

The proposed assumptions are in line with the concept of neuroendocrine-immune complex [13,25].

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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.

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