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RUDENKO A.V.[1], ROMANENKO A.M.[1], PASIECHNIKOV S.P.[1], MITCHENKO M.V.[1], ROMASHCHENKO O.V.[1*], TARADIY N.M.[2]

[1] State Institution «Academic O. F. Vozianov Institute of Urology of National Academy of Medical Sciences of Ukraine», 9a V. Vynnychenka Str., Kyiv, 04053, Ukraine

[2] International Center for Astronomical and Medico-Ecological Research of NAS of Ukraine, 27 Akademika Zabolotnoho Str., Kyiv, 03680, Ukraine

*Author for correspondence; e-mail: miclabor@gmail.com

DISORDER OF THE MICROBIOTA AND MUCOSAL IMMUNITY OF THE GENITAL TRACT IN WOMEN OF REPRODUCTIVE AGE WITH DIFFERENT CLINICAL COURSES OF ACUTE UNCOMPLICATED PYELONEPHRITIS

The research deals with the study of immuno-microbiological parallels of the genital tract in women with acute uncomplicated pyelonephritis with concomitant chronic pelvic inflammatory diseases. In addition to etiotropic antibacterial therapy, correction of the mucosal immunity state of the genital tract is crucial for the treatment of the disease and prevention of its recurrence. The ***purpose*** *of the work was to investigate the contamination and state of local immunity of the genital tract in women with acute uncomplicated pyelonephritis with different clinical courses.* ***Methods.*** *246 women of reproductive age suffering from acute uncomplicated pyelonephritis were studied, divided into three variants of the disease clinical course: group 1 — mild (n=105), group 2 —moderate (n=90), and group 3 — severe (n=51) courses. The scrapings of the mucous membrane of the cervical canal and washings from the vagina were obtained before the start of antimicrobial therapy. Quantitative determination of pathogens was carried out by sowing the biological material on solid nutrient environment. Identification of detached bacteria was carried out according to Bergey's. Mollicutes (Ureaplasma spp., Mycoplasma hominis) were determined by the cultural-fermentative method using test systems and the polymerase chain reaction. Vaginal washings of 121 sick women were used for immunological studies. The levels of myeloperoxidase, lysozyme, human β-defensin-2, immunoglobulins M, A, G, secretory IgA, lactoferrin, C3-component of complement, secretory component, and tumor necrosis factor-α were determined. The reference group consisted of 23*

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clinically healthy women. The Statistica 12.0 program package for Windows was used, and a difference of p<0.05 was considered verifiable. ***Results****. In the majority of patients with acute uncomplicated pyelonephritis (85.6% of cases), concomitant chronic pelvic inflammatory diseases (colpitis, salpingitis, underlying medical condition of cervix) were observed. Classical bacteria were more often detected in vaginal washings of patients of all groups, while mollicutes were detected in scrapings of the mucous membrane of the cervical canal, whereas the frequency of their detection increased with increase in the pyelonephritis severity. The analysis of indicators of local immunity determined disorders of mucosal immunity of the genital tract in the form of increased levels of myeloperoxidase, IgA, and IgG relative to reference values. The highest levels of lysozyme and myeloperoxidase were determined in patients with a severe course of acute uncomplicated pyelonephritis (by 5.3 and 3.6 times more, respectively), and the content of immunoglobulins A, M, and G in patients with a mild course of the disease (by 3.2, 3.1, and 4.0 times more, respectively). An increase in the level of tumor necrosis factor-α was detected in all patients, although no significant differences from controls were found in any group, but the highest median value was recorded in patients with severe pyelonephritis. In the group of patients with a severe clinical course, the lowest median was observed for β-defensin-2, which makes it impossible to inhibit the synthesis of TNF-α and, thus, supports the inflammatory process.* ***Conclusions.*** *It has been proven that in women with acute uncomplicated pyelonephritis, the severity of the course probably correlates with infection of the genital tract by mollicutes, mainly in association with classical bacteria and with level of mucosal immunity disorder. The obtained results of microbiological and immunological studies of biological material collected before the start of antibacterial therapy in patients with acute uncomplicated pyelonephritis proved the need for a mandatory examination by a gynecologist to ascertain concomitant chronic pelvic inflammatory diseases and establish infection with mollicutes to provide relevant etiotropic treatment. The above is the basis for adding the data regarding laboratory examination and treatment to the management protocols of patients with acute uncomplicated pyelonephritis.*

Keywords: *acute uncomplicated pyelonephritis, chronic pelvic inflammatory diseases, microbiological diagnosis, mollicutes, local immunity of the genital tract.*

The relevance of urinary tract infections, including acute uncomplicated pyelonephritis (AUP), is determined not only by their significant frequency but also by the difficulties in the etiological diagnosis, unfavorable course, and prognosis, with the prevalence of women of reproductive age among patients in this category constantly increasing. This is connected both with the peculiarities of the embryogenesis of the urinary and genital tracts of a woman and with their anatomical and physiological relationships under normal and pathological conditions. However, the question of the sources and ways of infection of the urinary tract and kidneys remains insufficiently studied [1, 2].

Particular difficulties arise when AUP in patients occurs amid chronic pelvic inflammatory diseases (CPID) and has a recurrent course [3, 4]. Previous studies have proven that CPID can be a constant infection source of the urinary tract and kidneys due to the translocation of the pathogen both by the ascending route and hematogenously or lymphogenously [5]. It should be emphasized that there is a significant «rejuvenation» of inflammatory processes of the pelvic organs in women, and not only classical bacteria but also mollicutes (mycoplasmas and ureaplasmas) and chlamydia, with tropism to the mucous membranes of both the urinary and genital tracts, are considered as etiological factors [6–8]. It becomes clear that the lack of professional sanitation of the genital tract by a gynecologist makes it impossible to effectively prevent recurrences of urinary tract infections and poses a threat of repeated ascending infection of the urinary bladder and kidneys [9, 10].

The studies of the state of local immunity of the urinary and genital tracts mucous membranes of women in clinical conditions have proved the presence of significant disorders in the latter, which should be considered one of the most important pathogenetic chains of the development and recurrence of AUP [11, 12]. The mucous membranes of the urinary tract (UT),

vagina, and cervix are inhabited by various microorganisms that are in constant antagonism or synergy. The colonization resistance of the vagina and mucous membranes of UT ensures the stability of the natural microbiocenosis, prevents colonization by pathogenic microorganisms and the active reproduction of opportunistic microflora. The mucous membrane of the urinary bladder exhibits a certain resistance to infectious agents due to the presence of antibacterial mechanisms, which are constantly active in healthy women, such as low pH value, high urea concentration, anti-adhesive mucopolysaccharide layer on the surface of the epithelium, bacterial growth inhibitors, immunoglobulins, and antimicrobial peptides [13, 14]. One of the factors of the protracted course of the inflammatory process in the kidneys and genitals in women and the frequent recurrence of diseases is the failure of the macroorganism protective systems, which is manifested in the disorder of the cellular and humoral links of immunity and a decrease in the indicators of non-specific resistance [15].

There is no doubt that solving the problem of infectious and inflammatory diseases of both the urinary and genital systems in women of reproductive age lies in the area of combining the efforts of all relevant specialists — urologists, gynecologists, microbiologists and immunologists.

The **purpose** of the work was to investigate the contamination and state of local immunity of the genital tract in women with acute uncomplicated pyelonephritis with different clinical courses.

Materials and Methods. 246 women (average age 25.6 ± 2.4 years), patients with AUP, treated in the Inflammatory Diseases Department of the State Institution «Academic O. F. Vozianov Institute of Urology of National Academy of Medical Sciences of Ukraine» were studied. Prior to enrolment in the study, patients provided informed consent to clinical trials with usage of their biological material. According to the results of the examination of patients by a gynecologist before admission to the hospital, 211 (85.8%) of the examined patients were found to have several concomitant chronic pelvic inflammatory diseases. Anamnestically, at least two STD risk factors were confirmed in all patients: lack of barrier contraception, a new sexual partner in the last three months, unmarried, young sexually active age (85.0% of examined patients were under the age of 30). Most patients (54.9%) had at least two of the following symptoms: the appearance or change of vaginal secretions, an unpleasant odor from the genitals, itching, pain during or after intercourse (dyspareunia). Dominant diseases were: colpitis — in 69.1% (n = 170), cervical erosion — in 49.6% (n = 122), and chronic salpingitis — in 32.9% (n = 81) of patients. Endocervicitis was diagnosed in 19.9% (n = 49) of the examined women.

For convenience of clinical picture assessment of the acute infectious inflammatory process in the kidneys, a division into three variants of the clinical course of disease was applied: group 1 — mild (n = 105), group 2 — moderate (n = 90), and group 3 — severe (n = 51). The mild course was characterized by pronounced symptoms within 1—2 days after hospitalization, positive dynamics took place from the first-second day of treatment. The moderate course, in addition to the duration of the marked symptoms for more than 2 days, was accompanied by moderately marked symptoms of intoxication (nausea, dry mouth, thirst, tachycardia), a moderate disorder of the leukogram within the range of leukocytosis up to $15.0x10^9$/L, an increase in the proportion of stab neutrophils up to 10%. The severe course was accompanied by sharply marked symptoms of intoxication: hypotension, vomiting, oliguria with the duration of the marked symptoms for more than 5 days and marked leukogram abnormalities (leukocytosis more than $15.0x10^9$/L, stab neutrophils more than 10%).

The materials for microbiological and immunological diagnosis were scrapings of the mucous membrane of the cervical canal (SMMCC)

and washings from the vagina, obtained before the start of antimicrobial therapy. Quantitative determination of pathogens was carried out by sowing biological material on solid nutrient environment — agars: blood, meat-peptone, yolk-salt, Endo or Levin, Saburo according to Rodoman (manufacturers: Farmaktyv (Ukraine), Himedia (India), Sanimed International Impex (Romania)). Identification of detached bacteria was carried out according to Bergey's [16]. Contamination by bacteria was assessed by the microbial count (in colony-forming units/mL), regardless of its value. The determination of mollicutes (*Ureaplasma* spp., *M. hominis*) was carried out simultaneously by the culture-fermentative method (CFM) using test systems from the Biomerieux company (France) and by the polymerase chain reaction (PCR) using primers and equipment from the Seegene company (Republic of Korea). Mollicutes are bacteria, but their properties and sizes are significantly different from other pathogens named opportunistic pathogenic bacteria, so the term «classical bacteria» is used to indicate their different taxonomic affiliation.

For immunological studies, washings from the vagina of 121 sick women were used. The levels of myeloperoxidase (MPO), lysozyme, human β-defensin-2 (hBD-2) (Immunodiagnostik, Germany), immunoglobulins (Ig) M, A, and G, secretory IgA (sIgA), lactoferrin (LF), C3 component of the complement (C3), secretory component (SC), tumor necrosis factor-α (TNF-α) (ICL.Inc., Abcam, Cell Sciences, Elabsccience, USA) were determined. The unification of the results was provided by the calculation of the established concentration of the indicator per 1 mg of protein in the studied biomaterial. To determine the protein concentration, the Bradford method and a calibration curve based on human albumin protein (Sigma-Aldrich, Germany) were used [17]. The reference group consisted of 23 clinically healthy women who did not express urological and gynecological complaints. Examination of them proved the absence of infection of the urinary and genital tracts with classical bacteria or mollicutes and chlamydia.

The research results were processed statistically using the Statistica 12.0 program for Windows. Calculations of the relative index (P) and its error (m) were carried out for nominal data. For quantitative variables, calculations of median (Me), lower and upper quartiles (25—75%) were used [18]. The data in independent groups were compared using the non-parametric Wilcoxon-Mann-Whitney U-test. The nominal data were compared using conjugation tables along with the calculation of the non-parametric Pearson chi-square test ($\chi 2$) with the number of degrees of freedom (df). To establish the bond strength between nominal variables for multi-field tables, Cramer's V -value was used, which varied from 0 to 1. A significance level of $p<0.05$ was considered reliable for all statistical analysis procedures.

Results. As a result of microbiological and molecular genetic studies, pathogens DNA of mollicutes were isolated and identified in the genital tracts of 208 women (84.5%): in 185 patients (75.2%) from scrapings of the mucous membrane of the cervical canal and in 122 patients (49.6%) — simultaneously with washings from the vagina. The following opportunistic pathogenic (classical) bacteria were isolated and identified: *Escherichia coli, Enterobacter cloacea, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis, Enterococcus faecium, Acinetobacter* spp., *Streptococcus* spp., *Streptococcus* α*-haemolyticus, Streptococcus* β*-haemolyticus, Staphylococcus aureus, Staphylococcus haemolyticus, Staphylococcus epidermidis,* and *Staphylococcus saprophyticus.* Classical bacteria were detected in samples in monoculture — in 35 (14.2%) patients, mollicutes in monoculture — in 110 (44.7%), and in association with bacteria — in 56 (22.8%) patients.

Candida fungi were identified in monoculture in 8 patients (3.2%) and in associations — in 36 women (14.6%).

The results of microbiological diagnosis of patients were analyzed depending on the clinical course variant (mild, moderate, or severe) of acute pyelonephritis. Classic bacteria, including *E. coli*, were isolated with a greater frequency in vaginal washings than in scrapings of the mucous membrane of the cervical canal for all groups of patients with AUP (Table 1).

The frequency of indication of fungi of the genus *Candida* spp. reliably prevailed in washings from the vagina in patients with a mild and moderate clinical course in comparison with scrapings of the mucous membrane of the cervical canal.

Mollicutes, in particular *Ureaplasma* spp., were more common in SMMCC of all patients groups. With increasing severity of the course of the inflammatory process, an increase in the frequency of detection of mollicutes was observed, which acquired statistically significant differences (Table 2).

According to the comparison of the general contamination of the genital tract in patients with different AUP using the table of mutual conjugation and Pearson's χ^2-square (with a significance level of $p < 0.05$), the existence of correlation was established between the frequency of detection of pathogens of different taxonomic affiliations in the genital tract and the severity of the course of pyelonephritis ($\chi^2 = 26.96$; $df = 6$; $p < 0.001$; the strength of the relationship

Table 1. **The species spectrum of classical bacteria and fungi of the genus Candida in the biological material of patients depending on the clinical course of AUP**

Pathogens	Frequency of detection of pathogens in the genital tract			
	in SMMCC		in washings from the vagina	
	n (patients)	P (%) ± m	n (patients)	P (%) ± m
Mild clinical course of AUP (group 1)				
	105		104	
Bacteria, including	18	17.1 ± 3.7	34	32.7 ± 4.6 *
E. coli	10	9.5 ± 2.9	23	22.1 ± 4.1 *
Fungi of the genus *Candida* spp.	4	3.8 ± 1.9	15	15.4 ± 3.5 *
Moderate clinical course of AUP (group 2)				
	90		89	
Bacteria, including	12	13.3 ± 3.5	33	37.1 ± 5.1 *
E. coli	7	7.8 ± 2.8	22	24.7 ± 4.6 *
Fungi of the genus *Candida* spp.	1	1.1 1.1	11	12.4 ± 3.5 *
Severe clinical course of AUP (group 3)				
	50		50	
Bacteria, including	9	18.0 ± 5.4	19	38.0 ± 6.9 *
E. coli	2	4.0 ± 2.8	8	16.0 ± 5.2
Fungi of the genus *Candida* spp.	3	6.0 ± 3.4	5	10.0 ± 4.6

* $p < 0.05$ comparing with SMMCC

is «relatively strong» according to Cramer's test (V = 0.234)). Statistically significant differences were established for patients with mild AUP compared to patients with severe AUP and moderate severity, regarding both classical bacteria in monoculture and ones in association with mollicutes in the genital tract (Table 3).

The number of patients whose genital tracts were infected only with mollicutes remained at an almost equally high level. A significant increase in the frequency of detection of bacteria and mollicutes associations was observed in patients of groups 2 and 3 compared to patients of group 1. Therefore, the severity of the AUP clinical course was associated with an increase in the mollicutes detection frequency in the genital tract both in monoculture and in association with classical bacteria: in group 1, they were detected in 52.4% of cases, in group 2 — in 75.6%, and in group 3 — in 84.3%.

At the same time, an analysis of the local immunity indicators in the vaginal washings of patients with AUP was performed depending on the VCC, which demonstrated the greatest increase (by 3.6 times) in the median values for myeloperoxidase (MPO) in patients of groups 2 and 3 compared to the group of healthy women (Table 4).

Table 2. **The frequency of mollicutes detection in the biological material of patients depending on the clinical course of AUP (according to two methods CFM + PCR)**

Groups of patients	Frequency of detection of pathogens in the genital tract			
	in SMMCC		in washings from the vagina	
	n/n+	P (%) ± m	n/n+	P (%) ± m
Group 1	105/55	52.4 ± 4.9	28/5	17.8 ± 7.2 *
Group 2	90/68	75.6 ± 4.5 ∇	8/4	50.0 ± 18.9
Group 3	50/43	86.0 ± 5.0 ∇	11/7	63.6 ± 15.2 ∇

n — number of samples; n+ — number of samples positive for mollicutes; *p < 0.05 comparing with SMMCC; ∇ — p <0.05 comparing with patients with mild AUP.

Table 3. **Contamination of the genital tract in patients (n=246) depending on the clinical course of AUP, n (%)**

Pathogens	Groups of patients with AUP			χ^2 Pearson's (df=1), p		
	Group 1 (n =105)	Group 2 (n=90)	Group 3 (n=51)	Gr. 1 — Gr. 2	Gr. 1 — Gr. 3.	Gr. 2 — Gr. 3
Bacteria in monoculture	24 (22.8)	9 (10.0) ∇	2 (3.9) ∇	χ^2 = 5.70 p = 0.017	χ^2 = 8.86 p = 0.003	χ^2 = 1.67 p = 0.196
Bacteria + mollicutes	12 (11.4)	25 (27.8) ∇	19 (37.3) ∇	χ^2 = 8.42 p = 0.004	χ^2 = 14.38 p<0.001	χ^2 = 1.36 p = 0.243
Mollicutes in monoculture	43 (41.0)	43 (47.8)	24 (47.0)	χ^2 = 0.64 p = 0.422	χ^2 = 0.52 p = 0.470	χ^2 = 0.002 p = 0.964
Bacteria and mollicutes not detected	26 (24.8)	13 (14.4)	6 (11.8)	χ^2 = 3.22 p = 0.072	χ^2 = 3.56 p = 0.059	χ^2 = 0.20 p = 0.654

∇ — p<0.05 comparing to patients with mild AUP

A probable increase in the level of MPO was established in patients with moderate severity of AUP compared to the mild course of AUP. The level of lactoferrin was lower than in control in patients of groups 2 and 3, even the median for LF in patients of group 2 reached a significant difference from this indicator in group 1.

Another protein with antibacterial action, lysozyme, had a significantly increased level in regard to the control in patients with severe AUP (by 5.3 times), in patients with mild and moderate courses, only a tendency to increase of its level was detected. The median for lysozyme in patients of group 3 was probably increased (by 3.0 times) compared to group 1.

The median for the activation indicator of the complement system (C3) in all groups had no significant difference compared to the control, but the interquartile range in patients with a severe course of the inflammatory process was wider, which indicates an increase in the number of patients with high values of the indicator.

Table 4. **Levels of humoral factors of local immunity in washings from the vagina of patients depending on the clinical course of AUP (n=121)**

Indicator (per 1 mg of protein)	Healthy women (n = 15)	Levels of humoral factors (*Me* / 25% — 75%)		
		Patients with AUP		
		Group 1 (n = 50)	Group 2 (n = 47)	Group 3 (n = 24)
MPO, mcg	1.7 0.88 — 3.50	1.02 * 0.28 — 3.80	6.10 * Ñ 3.37 — 8.97	6.15 * 2.50 — 11.60
LF, mcg	10.7 6.48 — 12.10	12.9 6.4 — 27.5	7.1 Ñ 2.8 — 14.4	8.3 5.1 — 24.0
Lysozyme, mcg	0.86 0.44 — 1.72	1.52 0.56 — 0.24	2.04 1.70 — 5.45	4.57 * Ñ 3.97 — 5.22
hBD-2, mcg	0.201 0.10 — 0.47	0.198 0.03 — 0.64	0.330 0.18 — 0.47	0.052 0.02 — 0.38
C3, mcg	0.29 0.19 — 0.40	0.41 0.11 — 0.67	0.28 0.12 — 0.60	0.52 0.12 — 1.18
sIgA, mcg	20.4 11.2 — 27.7	37.8 * 21.8 — 89.8	36.1 11.3 — 89.4	44.4 13.0 — 86.5
SC, mcg	19.0 13.0 — 26.5	21.4 6.9 — 35.5	15.8 8.0 — 28.6	16.9 6.8 — 33.1
IgA, mcg	34.0 26.3 — 40.5	109.0 * 50.0 — 200.0	67.0 * 28.0 — 176.1	91.0 * 29.0 — 275.1
IgM, mcg	3.25 2.20 — 4.60	10.2 * 4.9 — 15.5	7.8 1.8 — 11.7	6.4 3.6 — 11.5
IgG, mcg	53.9 37.1 — 77.2	213.8* 121.2 — 483.6	134.6* Ñ 70.0 — 307.0	157.1* 96.2 — 300.0
TNF- a, pg	33.8 23.3 — 174.7	46.9 24.5 — 105.0	59.2 19.5 — 161.4	110.3 14.6 — 266.2

*statistically significant differences compared to the group of healthy women; Ñ — for patients with mild AUP

Immunoglobulins of the IgA class, especially its secretory form — sIgA, are of crucial importance in providing local immunity of the vaginal mucosa. A significantly increased level of sIgA (by 1.8 times) relative to the control value was recorded only in patients with mild AUP. A slightly wider interquartile range of sIgA values was observed in patients of the other two groups. The median and interquartile range for the secretory component in vaginal washings of patients with different AUP varied within the control range, which should be considered insufficient to protect against infection in conditions of genital tract infection.

A significantly increased level of IgA and IgG in all groups of patients with different VCC compared to the indicators of the control group was noted. In patients with a mild course of the disease, the highest median values for immunoglobulins A, M, and G were found (by 3.2, 3.1, and 4.0 times relative to control values). A significant difference was also documented for the level of IgG between patients in groups 1 and 2.

In response to infection and the development of the inflammatory process, cytokines are synthesized locally, in particular TNF-α. During the study of vaginal washings in patients with AUP, a tendency to increase in the level of TNF-α comparing to the control index was recorded, to a greater extent in patients with severe AUP (by 3.3 times compared to the control).

Discussion. The causative agents of chronic inflammatory diseases of the female reproductive tract are aerobic-anaerobic associations, including causative agents of sexually transmitted infections and opportunistic pathogenic representatives of the normal microflora of the vagina [19, 20]. Therefore, changes in the vaginal microbiocenosis with an increase in the number of potential pathogens are one of the risk factors for the occurrence of the female reproductive tract diseases [21, 22]. On the other hand, acute and chronic inflammatory processes of the pelvic organs, taking into account their influence on the hormonal and immune status of the body, can lead to disturbances in the composition of the normal vaginal microflora [23]. In addition, the anti-infective resistance of the vaginal biotope is mainly determined by factors of local immunity [24, 25].

On the basis of the conducted research, it can be stated that in patients with acute pyelonephritis amid CPID, changes in the humoral factors of local immunity were observed, and their severity was correlated with the taxonomic affiliation of the pathogen and the clinical course of the disease.

Dysbacteriosis increases the number of pathogens, potentially triggering the secretion of antimicrobial peptides. A number of authors have emphasized the importance of antimicrobial peptides as an indispensable component in the control of infectious and inflammatory processes [26–28].

Thus, the most significant increase in the levels of such bactericidal peptides as myeloperoxidase and lysozyme was observed in examined patients with a severe course of the disease, which indicates a significant degree of inflammation in conditions of massive infection. A probable increase in MPO levels was found in patients with the moderate course compared to the mild AUP. Myeloperoxidase forms a more powerful bactericidal system together with lactoferrin, since the mutually reinforcing effect of the MPO system and lactoferrin has been proven. The observed increase in MPO levels in patients with severe and moderate severity of AUP should be considered a compensatory reaction due to the fact that lactoferrin levels in severe and moderate severity of the disease were lower than in the control, which is insufficient in case of genital tract infection. Even the median value for LF in patients of group 2 reached a significant difference from this indicator in group 1.

Antimicrobial peptides are the first line of defence of mucous membranes against pathogenic

bacteria, so an excess of antimicrobial peptides in the epithelial microenvironment is associated with inflammation and its severity [29]. It is known that lysozyme exhibits synergism with defensins, positively charged amino acids bound electrostatically to the cytoplasmic membranes of pathogens [30]. β-Defensins play an important role in protecting female genital tract from pathogenic bacteria and fungi. The synthesis of the latter occurs in mucosal epithelial cells when they are stimulated by lipopolysaccharides of the microbial cell wall [31, 32]. The study proved that the levels of β-defensin-2 in patients of different groups were within the reference values, but in patients with the severe clinical course, the lowest levels were detected. This fact may be due to the presence of «atypical» pathogens — mollicutes, which lack a cell wall and have only a cell membrane. Mollicutes prevailed in microbial associations in patients with severe AUP. In addition, complement activation was subsidiarily observed due to an increase in the C3-component of complement in many patients of this group.

There are known studies that have shown that the main carrier of specific antiviral and antibacterial activity of secretions is secretory IgA, which inhibits the adhesion of microorganisms and their toxins on the surface of the mucosal epithelium [20]. Antibodies of this class prevent the occurrence of pathological processes on the mucous membrane without causing its trauma, since the interaction of sIgA-antibody with antigen, unlike antibodies of lgG and IgM classes, does not cause activation of the complement system [21, 22].

The conducted study verified that the level of secretory immunoglobulin A is insufficient to protect the genital tract from infection; only in patients with a mild course of the disease it is significantly different from the norm. The levels of immunoglobulins of other classes (A, M, G) were elevated in patients of all groups, which can be considered another criterion of the inflammatory process that occurred in the genital tract in patients with acute pyelonephritis amid CPID.

It is known that TNF-α in the inflammatory center increases the cytotoxicity of macrophages and monocytes [24]. The pattern of the inflammatory response of the examined patients is complemented by the tendency to an increase in the level of this proinflammatory cytokine in vaginal washings from all groups of patients with the highest value in women with severe AUP. This fact should be explained by the insufficient level of β-defensin-2 in patients of group 3, which creates conditions for the development of an inflammatory reaction.

Thus, the state of the immunological reactivity of the macroorganism determines how successfully the effector links of humoral and cellular immunity will resist pathogenic and opportunistic pathogenic microorganisms and allow it to overcome the barriers of its immunobiological protection, the ability to colonize and persist in the body for a long time [33].

Evaluating the results of the study of the species spectrum of causative agents of the inflammatory process of the kidneys and genital tracts in women with acute uncomplicated pyelonephritis, and the proven superiority of mollicutes in the studied biomaterial, we consider it appropriate to cite an example of our experimental studies on rabbits concerning the pathogenicity of ureaplasmas by modelling ureaplasmosis of the kidneys and uterus. Thus, during surgery, animals were injected into the urethra (group I) and the right uterine horn (group II) with a suspension of ureaplasmas (*Ureaplasma parvum*) isolated from the urine and scrapings of the cervical canal of patients with AUP (0.5 mL at a concentration of 10^5 colony-forming units/mL). After 30 days, the animals were removed from the experiment. During the study of biomaterial (kidneys and uterus), ureaplasmas were isolated, and morphologically, regardless of which organ the ureaplasmas were injected into, changes were recorded that were charac-

terized by increased blood vessel filling, hemorrhages, swelling, lymphoid cell infiltration of the stroma, and dystrophic changes, necrosis, epithelial exfoliation [34, 35].

So far, we have established the tropism of *Ureaplasma* spp. to the mucous membranes of both the urinary and genital tracts. The obtained experimental results for modeling the inflammatory processes in the kidneys and uterus give a reason to assess the role of mollicutes as causative agents of the inflammatory process inthe kidneys and genitals with their corresponding pathomorphosis.

The obtained results of microbiological and immunological studies of biological material from female genital tract collected before the start of antibacterial therapy in patients with acute uncomplicated pyelonephritis proved the need for a mandatory examination by a gynecologist to ascertain concomitant chronic pelvic inflammatory diseases and establish infection with mollicutes to provide relevant etiotropic treatment. The above is the basis for adding the management protocols of patients with acute uncomplicated pyelonephritis [36].

Conclusions. It has been proven that the majority of patients with acute uncomplicated pyelonephritis (85.6% of cases) had concomitant chronic pelvic inflammatory diseases (colpitis, salpingitis, cervix erosion).

In patients with acute uncomplicated pyelonephritis, the course severity probably correlates with infection of the genital tract by mollicutes, mainly in association with classical bacteria. Classical bacteria were more often identified in vaginal washings of patients with different variants of the clinical course of acute uncomplicated pyelonephritis.

In all patients with acute uncomplicated pyelonephritis in the presence of concomitant chronic pelvic inflammatory diseases, disorders of mucosal immunity of the genital tract were determined in the form of increased levels of myeloperoxidase, IgA, and IgG relative to reference values.

The highest levels of lysozyme and myeloperoxidase were determined in patients with a severe course of acute uncomplicated pyelonephritis (by 5.3 and 3.6 times more, respectively) along with the content of immunoglobulins A, M, and G in patients with a mild course of the disease (by 3.2, 3.1, and 4.0 times more, respectively).

The tendency to an increase in the level of tumor necrosis factor-α was detected in all patients with acute uncomplicated pyelonephritis, although no significant differences from controls were found in any group, but the highest median value was recorded in patients with severe pyelonephritis. In the group of patients with a severe clinical course, the lowest median was observed for β-defensin-2, which reduces the antibacterial defense of the mucous membranes of the genital tract and causes support for the inflammatory process.

The obtained results of microbiological and immunological studies of biological material (scrapings of the mucous membrane of the cervical canal and washings from the vagina) in patients with acute uncomplicated pyelonephritis proved the need for a mandatory complex examination (urologist and gynecologist) and the use of appropriate etiotropic treatment of patients in the presence of pathogens such as mollicutes.

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Руденко А.В.[1], Романенко А.М.[1], Пасєчніков С.П.[1], Мітченко М.В.[1], Ромащенко О.В[1], Тарадій Н.М.[2]

[1] Державна установа «Інститут урології імені академіка О.Ф. Возіанова НАМН України», вул. В. Винниченка, 9а, Київ, 04053, Україна

[2] Міжнародний центр астрономічних та медико-екологічних досліджень НАН України, вул. Академіка Заболотного, 27, Київ, 03680, Україна

ПОРУШЕННЯ МІКРОБІОТИ ТА МУКОЗАЛЬНОГО ІМУНІТЕТУ СТАТЕВИХ ШЛЯХІВ У ЖІНОК РЕПРОДУКТИВНОГО ВІКУ З РІЗНИМ КЛІНІЧНИМ ПЕРЕБІГОМ ГОСТРОГО НЕУСКЛАДНЕНОГО ПІЄЛОНЕФРИТУ

Дослідження присвячено вивченню імуно-мікробіологічних паралелей статевих шляхів у жінок, хворих на гострий неускладнений пієлонефрит (ГНП) із супутніми хронічними запальними хворобами органів малого таза. Крім етіотропної антибактеріальної терапії, корекція стану мукозального імунітету статевих шляхів має вирішальне значення для лікування захворювання та профілактики його рецидивів. **Мета** роботи — дослідити інфікованість та стан місцевого імунітету статевих шляхів у жінок, хворих на ГНП з різним клінічним перебігом. **Методи.** Об'єктом дослідження були 246 жінок репродуктивного віку, хворих на ГНП, яких розподілили за варіантами клінічного перебігу ГНП: група 1— легкий (n=105), група 2 — помірний (n=90) та група 3— важкий (n=51). Зіскрібки слизової оболонки цервікального каналу та змиви з піхви одержували під час госпіталізації хворої до початку антимікробної терапії. Кількість збудників визначали шляхом посіву біологічного матеріалу на тверді поживні середовища. Ідентифікацію виділених бактерій проводили за Bergey's. Одночасно визначали молікутів (*Ureaplasma* spp., *Mycoplasma hominis*) культурально-ферментативним методом за допомогою тест-систем та шляхом полімеразно-ланцюгової реакції. Для імунологічних досліджень використовували змиви з піхви від 121 хворої жінки. Визначали рівні мієлопероксидази, лізоциму, b-дефензину-2 людини, імуноглобулінів M, A, G, секреторного IgA, лактоферину, C3-компонента комплемен**т**у, секреторного компонента, фактора некрозу пухлин-а. Референтну групу склали 23 клінічно здорові жінки. Використовували пакет програм Statistica 12.0 для Windows, достовірною вважали різницю з $p < 0.05$. **Результати.** У більшості хворих на ГНП (85.6% випадків) спостерігали супутні хронічні запальні хвороби органів малого тазу (кольпіт, сальпінгіт, ерозія шийки матки). Класичні бактерії частіше визначали у змивах із піхви хворих усіх груп, тоді як молікути — у зіскрібках слизової оболонки цервікального каналу, при цьому частота їх виявлення збільшувалася зі зростанням важкості перебігу ГНП. Аналіз показників місцевого імунітету визначив порушення мукозального імунітету статевих шляхів у вигляді збільшення рівнів мієлопероксидази, IgA, IgG відносно референтних значень. Найбільші рівні лізоциму та мієлопероксидази визначено у хворих за важкого перебігу ГНП (відповідно в 5.3

та 3.6 рази), а вмісту імуноглобулінів A, M, G — у пацієнток із легким перебігом захворювання (відповідно в 3.2, 3.1 та 4.0 рази). Спостерігали зростання рівня фактора некрозу пухлин-α у всіх хворих, хоча у жодній групі не було виявлено достовірних відмінностей від контролю, але у хворих з важким перебігом пієлонефриту зафіксовано найбільше значення медіани. У групі хворих з важким клінічним перебігом ГНП визначено найменшу медіану для β-дефензину-2, що унеможливлює пригнічення синтезу ФНП-α і, таким чином, спричиняє підтримку запального процесу. **Висновки**. Доведено, що у жінок, хворих на ГНП , важкість перебігу захворювання, вірогідно, корелює з інфікуванням статевих шляхів молікутами, переважно в асоціації з класичними бактеріями, та із ступенем порушень мукозального імунітету. Одержані результати мікробіологічного та імунологічного досліджень біологічного матеріалу, зібраного до початку антибактеріальної терапії у хворих на ГНП, довели необхідність обов'язкового огляду гінекологом для констатації супутніх хронічних запальних хвороб органів малого тазу та встановлення інфікування молікутами для застосування відповідного етіотропного лікування. Вищезазначене є підставою для доповнення до протоколів ведення хворих на ГНП лабораторного обстеження й лікування.

***Ключові слова**: гострий неускладнений пієлонефрит, хронічні запальні хвороби органів малого тазу, мікробіологічна діагностика, молікути, місцевий імунітет статевих шляхів.*