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Research Article

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Cytokines and SLPI profiles in children and adults with urinary tract infections

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Abstract. *Pro- and anti-inflammatory cytokines, SLPI and NGAL are involved in anti-infectious immunity. Studies of these indicators' role in patients with urinary tract infections (UTIs) may determine their significance as diagnostic and prognostic markers in the case of pyelonephritis and cystitis.*

The objective of our study was to investigate plasma and urine cytokines and SLPI levels in patients with UTIs, determine the features in children and adults.

Materials and methods. ELISA method and related test systems – «Immunotech», «Diaclon» (France); «DRG» (Germany), «Hycult biotechnology» ((Netherlands) were used to study blood cytokine levels in 118 adults and 67 children, SLPI levels in the blood and urine in 59 and 58, respectively. NGAL serum levels of 26 adults with acute pyelonephritis (AP) and 30 adults with chronic pyelonephritis (ChP) were studied using «Human lipocalin-2 / NGAL ELIZA» (Biovendor, Czech Republik) for NGAL. Comparison groups included 10 healthy donors and 11 patients with acute kidney injury (AKI).

Results. The study showed an increase in pro- (IL-1, -17, -18, -23, TNF- α , MCP-1) and anti-inflammatory cytokines (IL-17, TGF- β), SLPI, NGAL in patients with UTI, some features in the case of chronic cystitis (ChC), AP and ChP. In adults, TNF- α in the blood and urine, IL-17 in the blood was higher in the case of ChC than ChP. The analysis showed a significant increase in all studied indicators' levels for AP and ChP in children and adults. The average MCP-1 level in patients with AP was significantly higher than ChP, whereas TNF- α did not differ. In adults, IL-18 and IL-23 were highest in the case of AP, and TGF- β was the highest in the case of ChP. MCP-1, IL-23 levels in the blood of adults were higher than in children in the case of AP, and TNF- α - in the case of ChP. SLPI is involved in the AP pathogenesis and ChP exacerbation. High SLPI levels have been determined in serum and urine (NGAL in the blood) in patients with pyelonephritis (NGAL - AP) who can be used, as well as cytokines, as additional diagnostic and prognostic markers.

Conclusions. High levels of TNF- α , MCP-1, and IL-23 in the blood of adults and children confirm their important role in both AP and ChP, but MCP-1 can be considered as an AP predictor/ ChP exacerbation. According to the studied cytokines, adults have a more significant immune response. The SLPI level is an additional feature for diagnosing and monitoring the course of pyelonephritis and cystitis.

Keywords: urinary tract infections, pyelonephritis, cytokines, secretory leukocyte protease inhibitor.

Conflict of interest statement: all the authors declared no competing interests.

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Профіль цитокінів і SLPI у дітей і дорослих з інфекцією сечової системи

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Резюме. Про- і протизапальні цитокіни, SLPI і NGAL беруть участь в протийнфекційному імунітеті. Дослідження ролі цих показників у хворих на інфекції сечової системи (ІСС) можуть визначити їх значення в якості діагностичних та прогностичних маркерів у разі пієлонефриту і циститу.

Метою роботи було дослідити концентрацію цитокінів і SLPI у крові і сечі пацієнтів з ІСС, визначити особливостей у дітей та дорослих.

Матеріали і методи. Імуноферментний метод ELISA і відповідні тест-системи - «Immunotech», «Diaclon» (Франція); «DRG» (Німеччина), «Hycult biotechnology» (Нідерланди) були використані для вивчення рівнів цитокінів в крові у 118 дорослих і 67 дітей, рівнів SLPI в крові і сечі відповідно, у 59 і 58. Були вивчені рівні NGAL в сироватці крові 26 дорослих з гострим (ГП) і 30 – хронічним пієлонефритом (ХП) з використанням «Human lipocalin-2 / NGAL ELIZA» (Biovendor, Чехія) для NGAL, групи порівняння – 10 здорових донорів і 11 пацієнтів з гострим пошкодженням нирок (ГПН).

Результати. Дослідження показало збільшення рівня про- (IL-1, -17, -18, -23, TNF- α , MCP-1) і протизапальних цитокінів (IL-17, TGF- β), SLPI, NGAL у пацієнтів з ІСС, деякі особливості у разі хронічного циститу (ХЦ), ГП та ХП. У дорослих TNF- α в крові і сечі, IL-17 в крові був вище у разі ХЦ, ніж ХП. Аналіз показав достовірне збільшення рівнів всіх вивчених показників при ГП і ХП у дітей і дорослих. Середній рівень MCP-1 у пацієнтів з ГП достовірно вище, ніж ХП, тоді як TNF- α не відрізняються. У дорослих IL-18 і IL-23 були найвищими у разі ГП, а TGF- β - ХП. Рівні MCP-1, IL-23 в крові у дорослих були вище, ніж у дітей у разі ГП, а TNF- α - ХП. SLPI бере участь в патогенезі ГП та загострення ХП. Високий рівень SLPI встановлений в сироватці і сечі (NGAL в крові) у пацієнтів з пієлонефритом (NGAL - ГП), які можуть бути використані, як і цитокіни, в якості додаткових діагностичних і прогностичних маркерів.

Висновки. Високий рівень TNF- α , MCP-1 і IL-23 в крові у дорослих і дітей підтверджує їх важливу роль як при АР, так і при ПП, але MCP-1 можна розглядати як предиктор ГП/загострення ХП. За даними вивчених цитокінів, у дорослих відзначений більш виражений імунну відповідь. Рівень SLPI є додатковою ознакою для діагностики і стеження за динамікою перебігу пієлонефриту і циститу.

Ключові слова: інфекції сечової системи, пієлонефрит, цитокіни, секреторний інгібітор лейкопротеаз.

Background: Cytokines are key factors in the pathological process, including the urinary system diseases [1-3]. The urinary tract mucous membrane contains numerous protective effector molecules, including antimicrobial peptides and protease inhibitors, such as secretory leukocyte inhibitors (SLPIs), produced by mucous cells, macrophages and neutrophils, which help protect against elastases that are secreted by activated neutrophils and have antibacterial anti-inflammatory properties [5-6]. Plasma and urine cytokines and SLPI levels in patients with chronic urinary tract infections, including cystitis and pyelonephritis, can play a significant role in the disease pathogenesis.

Animal tests have shown that NGAL (neutrophil gelatinase-associated lipocalin) is one of the earliest

proteins that are induced in the kidney during ischemic or nephrotoxic stress. It can take part in antibacterial protection due to binding to bacterial siderophores. NGAL is produced by immunocompetent cells, tubule cells, hepatocytes, etc.

The **objective** of our study was to investigate the cytokines and SLPI levels' features as urinary tract infections' (UTIs) immunogenesis participants in children (average age 7.4 ± 0.6) and adults (average age 36.5 ± 8.2); serum NGAL levels in patients with acute (AP) and chronic pyelonephritis (ChP) and their correlation with pro-inflammatory TNF- α .

Methods: IL-1 β , -17, -18, -23, TNF- α , MCP-1, TGF- β levels in the peripheral blood of 12 adult patients with chronic cystitis - group 1 (gr), 55 patients with chronic pyelonephritis (2 gr) and 51 patients with acute pyelonephritis (3 gr) were determined by ELISA using SunRise TouchScreen ("Immunotech", "Diaclon" (France); "DRG" (Germany). Blood and urine SLPI levels were determined in 12 patients of 1 gr, 30 - 2 gr and 17 - 3 gr using "Hycult biotechnology" for Human SLPI (Netherlands).

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MCP-1, IL-23, TNF- α peripheral blood levels were evaluated in 35 children with chronic pyelonephritis (2nd gr) and in 32 children with acute pyelonephritis (3rd gr). Blood and urine SLPI levels were assessed in 26 children with ChP in remission (group I) and in 12 patients with ChP in the acute stage (group II), as well as in 20 patients with AP (group III). Normal levels of cytokines and SLPI were determined by examining 25 healthy adults and 10 children.

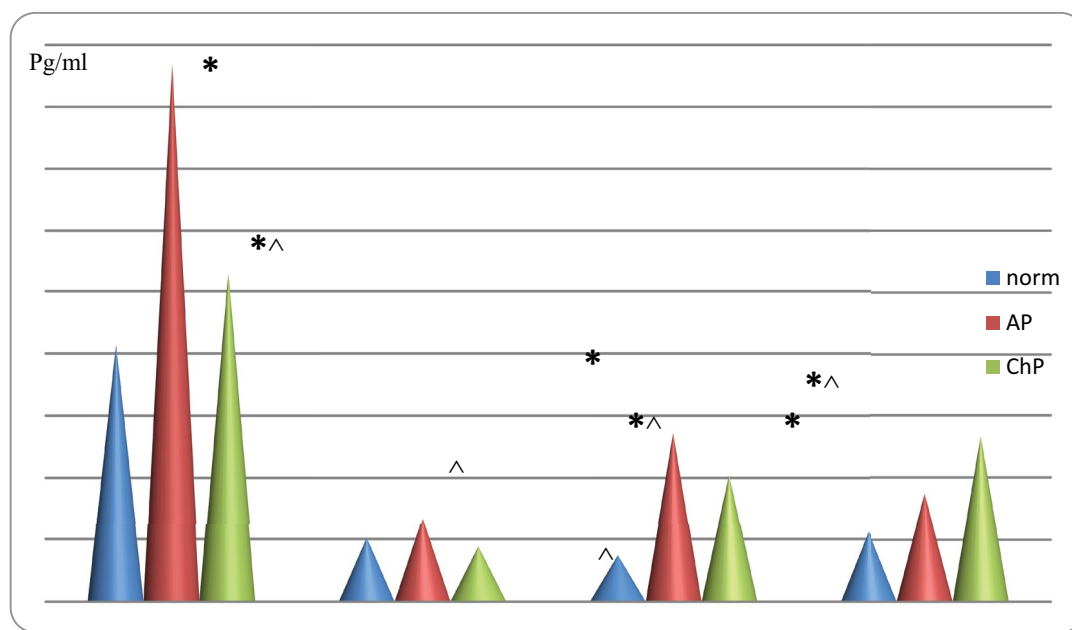
There were studied the NGAL levels in the blood serum of 26 adults with acute (group 1) and 30 with chronic pyelonephritis (group 2). As the control group was used 10 healthy donors and 11 patients with acute kidney injury (AKI) prerenal, renal and postrenal genesis in the oliguric stage and diuresis restoration stage (polyuric). It was used ELIZA and "Human lipocalin-2/NGAL ELIZA" (Biovendor, Czech Republik) for NGAL. Statistical analysis was performed with SPSS for windows 11,0 software package.

Serum NGAL levels were studied in 26 adults with acute (group 1) and 30 with chronic pyelonephritis (group 2). As a control group, we examined 10 healthy

donors and 11 patients with acute kidney injury (AKI) of prerenal, renal and postrenal genesis in the oliguria stage and the diuresis (polyuria) restoration stage. ELIZA and "Human lipocalin-2 / NGAL ELIZA" (Biovendor, Czech Republik) for NGAL were used to determine NGAL. Statistical analysis was performed using SPSS for the Windows 11.0 software package.

Results: Patients with chronic UTIs in both groups showed a high level of cytokines compared to normal healthy donors ($p < 0.05$). Serum TNF- α levels were higher in group 1 - 119.6 ± 6.5 compared with group 2 - 86.5 ± 2.0 pg/ml ($p < 0.05$), urine levels in the 1st group exceeded levels in the 2nd group in 3 times ($p < 0.001$). Serum IL-17 levels were also higher in the 1st group - 145.5 ± 2.0 versus 113.7 ± 3.1 in the 2nd group ($p < 0.05$). IL-1, MCP-1, TGF- β did not differ between group 1 and group 2 ($P > 0.05$), although they exceeded normal levels.

When comparing group 2 and group 3, MCP-1 ($p < 0.001$), IL-18 ($p = 0.038$) and IL-23 ($p = 0.028$) were the highest in the acute process and TGF- β - in case of ChP ($p = 0.004$) (fig. 1).



* - the difference with the norm is significant; ^ - 2-3 gr difference is significant.

Fig. 1. T Median levels of pro (MCP-1, IL-18, IL-23) and anti-inflammatory (TGF- β) cytokines in the serum of patients with acute and chronic pyelonephritis.

The individual analysis showed that the MCP-1 level was higher than 350 pg/ml in 74% of patients with AP, while in 4.8% ($p < 0.001$) of patients with ChP exacerbation.

The difference between group 2 and group 3 in the IL-1 ($p = 0.774$), TNF- α ($p = 0.269$) and IL-17 ($p = 0.905$) median levels is not significant (Table 1).

Table 1

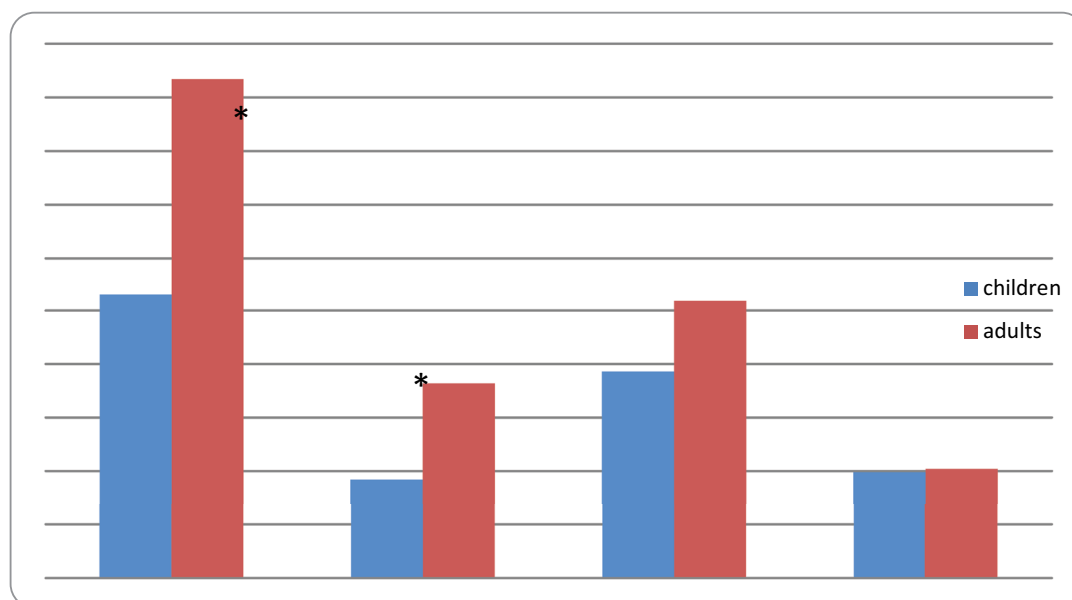
Median serum cytokine levels in patients and healthy donors

Indication (pg/ml)	Healthy donors	Patients with AP	Patients with ChP	P 3-2	P 4-2	P 3-4
IL-1	94.4 \pm 8.1	210.2 \pm 31.0	124.1 \pm 4.4	$p < 0.001$	$p < 0.001$	$p = 0.774$
TNF- α	22.4 [19.0; 24.0]	56.1 [35.3; 95.6]	73.9 [57.4; 92.3]	$p < 0.001$	$p < 0.001$	$p = 0.269$
IL-17	68.2 [37.9; 90.9]	121.3 [94.2; 163.5]	114.2 [89.2; 37.9]	$p < 0.001$	$p < 0.001$	$p = 0.905$

In children with AP, MCP-1 significantly exceeded the level with ChP ($p = 0.002$), whereas for TNF- α ($p = 0.562$) and IL-23 ($p = 0.339$), this difference was not significant.

It was important to conduct a comparative analysis of indicators in children and adults, noting that there

were no differences between healthy people of these age groups. Studies have shown higher levels of MCP-1 ($p < 0.001$) and IL-23 ($p = 0.039$) in adults with AP; there was no significant difference depending on age with ChP - respectively, $p = 0.758$ and $p = 0.805$ (Fig. 2); the mean values of TNF- α in these groups do not differ ($p \geq 0.05$).

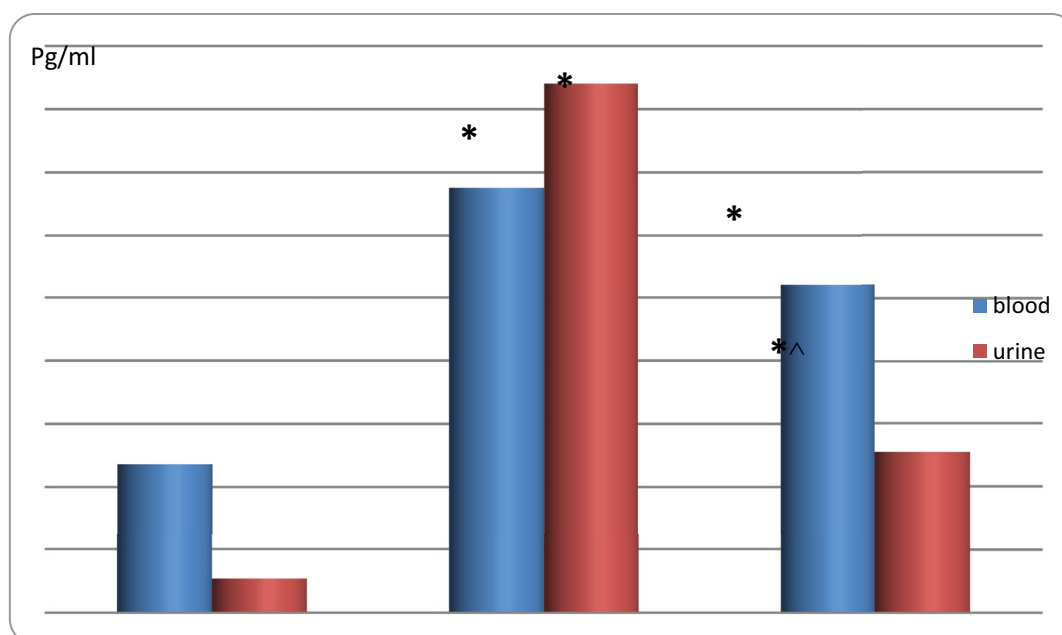


* - AP-ChP differences are significant

Fig. 2. Median serum levels of MCP-1 and IL-23 in children and adults with AP and ChP

The SLPI level in the urine of adults from group 1 was higher (4171 ± 225 pg/ml) than in group 2 (1857 ± 203) ($p < 0.05$). Serum SLPI levels of group 1 (2052 ± 378) and group 2 (2240 ± 178) were higher than normal (1181 ± 76) (Fig. 3), but did not differ between groups ($P > 0.05$).

The SLPI level was significantly increased in both forms of pyelonephritis ($p < 0.001$), but these values in the urine with AP were significantly higher (in 2.5 times, $p < 0.001$) than in the case of ChP; in these patients' blood, an upward trend was revealed ($p = 0.066$) (Fig. 3).



* - The difference with the norm is significant; ^ - difference 2-3 gr is significant

Fig. 3. Median serum SLPI levels in adults with AP and ChP compared with healthy donors

Statistically significant differences were noted between the SLPI concentration in children with AP (3454.7 [2911.0; 3740.9] in the blood and 150.7 [55.7; 316.0] in the urine) and ChP (3263, 9 [2395, 9; 3483.3] in the blood and 81.8 [40.0; 194.0] in the urine); and

also when comparing values during exacerbation (3235.3 [2314.8; 3554.9] and 178, 6 [48.8; 449.7]) and in remission (3397.5 [2677.3; 3421.3] and 81.0 [36.4; 170.7]) in patients with chronic kidney inflammation (Table 2).

Table 2

Serum and urine SLPI levels (pg/ml) in healthy children and patients of I-III groups

Groups		Mediana (pg/ml)	25%	75%	Min.	Max.
I gr	blood	3397,5*	2677,3	3421,3	2395,9	4012,7
	urine	81,0*	36,4	170,7	0,7	453,2
II gr	blood	3235,3*	2314,8	3554,9	1099,5	4327,5
	urine	178,6*	48,8	449,7	26,8	750,8
III gr	blood	3454,7*	2911	3740,9	1099,5	4327,5
	urine	150,7*	55,7	316	24,6	750,8
Healthy donors	blood	1741	1618	1874	929,6	1966
	urine	35,6	23,5	40,8	13,8	45,1

* $p \leq 0,05$ with N, p_{I-II} , p_{II-III} , $p_{I-III} \geq 0,05$

There is a correlation between the SLPI concentration and the number of lymphocytes in the peripheral blood ($p < 0.05$) in patients with AP ($r_K = 0.605$) and with ChP in the acute stage. A correlation was found between SLPI levels in blood and IL-17, TGF- β (Tau = 0.527, $p = 0.03$) in adults of 2nd group; other relations in all groups between IL-1 β , -18, -23, TNF- α , MCP-1, as well as in all groups in children between pro-inflammatory IL-23 and MCP-1 and SLPI were not significant ($p > 0, 05$).

The median NGAL level exceeded the norm by 1.5 times in 56 adult patients with kidney infection: 17.8 [14.4; 22.4] and 13.2 [10.7; 18.4] ng / ml ($p = 0.032$). In group 3, NGAL was significantly higher than normal - 21.2 [13.3; 23.8] ($p = 0.027$), and in the 2nd group there was only a tendency to increase - 17.6 [14.4; 20.0] ng / ml ($p = 0.072$). There was no significant difference between the two groups ($p = 0.223$). Compared to the group with acute kidney injury (AKI), NGAL levels were lower in comparison with patients not only from the 2nd group ($p = 0.006$) but from the 3rd group ($p = 0.010$), too. There was no correlation between the NGAL and TNF- α levels in any group ($p > 0.05$).

Discussion. It is known that the inflammation process is regulated by two types of biologically active substances, some initiate and support inflammation, others reduce the severity of the process. In the early inflammatory process stages, the cytokine complex provides a common mechanism of process progression, which does not depend on the damaging agent nature [1, 4, 7]. Within a few hours after exposure to pathogens, tissue macrophages trigger the synthesis of pro-inflammatory cytokines (IL-1, TNF- α , etc.) in the focus of inflammation. They are the cause of many local and systemic changes in the acute inflammatory response develop-

ment. A complex of local protective responses is activated under their action, involving almost all types of inflammatory effector cells to eliminate the pathogen and restore the damaged tissue integrity [7]. Therefore, the results of pro (IL-1, TNF- α , MCP-1, IL-17, and IL-23) and anti-inflammatory (TGF- β , IL-17) cytokine analysis are of scientific and practical interest.

Our studies showed that TNF- α in the blood and urine, IL-17 in the blood was higher in patients with chronic cystitis compared with patients with kidney inflammation. High levels of SLPI (> 3200 pg/ml) in urine were detected in the presence of cystitis. That is, cytokines and SLPIs play an important role in the bladder mucosa immunity and antibacterial immunity while the urinary system chronic infections.

We showed a significant increase in the median levels of pro-inflammatory cytokines TNF- α , MCP-1 and IL-23 in the blood of patients with AP and ChP, both adults and children. The MCP-1 (also IL-23 in adults) level with AP is significantly higher than with ChP, while TNF- α is not different.

This can be explained by the known MCP-1 biological effects and its role in inflammation. So, MCP-1 is one of the key chemokines that regulate monocyte/macrophage migration and infiltration. It is produced by many types of cells, including, in addition to monocytes and macrophages, mast cells, T and B lymphocytes, osteo- and fibroblasts, astro- and melanocytes, stellate liver cells, endothelial cells, renal epithelium, mesangial cells, etc. MCP-1 synthesis is induced by lipopolysaccharides, interleukins (-1, -4, -6), TNF- α , IFN- γ [8]. High production of MCP-1, as well as other pro-inflammatory mediators, promotes the development of cell apoptosis, necrosis and the inflammation progression [9].

High levels of TNF- α , MCP-1 and IL-23 in the blood in adults and children confirm their important role in both AP and ChP, but MCP-1 can be considered as an indicator of acute / exacerbation of chronic pyelonephritis [10, 11].

High production of pro-inflammatory mediators is a normal immune system response to bacterial antigens, but the investigated cytokines can exacerbate the pathogenic effect by stimulating the profibrogenic TGF- β secretion (this growth factor was highest in patients with ChP). Nephrosclerosis development is influenced by cell apoptosis, which can be an additional risk factor for immunodeficiency development, as well as renal failure in chronic inflammation [5, 7, 9]. According to the investigated cytokines, a more evident immune response is observed in adults.

The study showed that serum SLPIs were higher in patients compared to normal and the highest in group 3, with a significant difference from patients in group 1 and group 2, whose indications were above normal but not different from each other ($P \geq 0.05$). Patient urinary SLPI was also higher than normal. High levels of SLPI in the urine confirm the diagnosis of cystitis and play an important role in the bladder mucosa immune response.

In recent years, it has been of interest to study such an indicator as NGAL, an increase in which in the blood and urine correlates with early renal dysfunction. NGAL is produced by immunocompetent cells, hepatocytes, renal tubule cells, and others with ischemic or

nephrotoxic stress, and can also participate in protection against bacterial infections by interacting with microbial siderophores [12].

Conclusions. Our investigation showed that serum NGAL levels were highest in patients with AP. This fact may confirm that renal dysfunction is more severe in acute kidney inflammation (not associated with high levels of TNF- α). Despite lower serum NGAL levels compared with acute renal failure, these data can be used as additional markers of the renal dysfunction severity in patients with pyelonephritis.

Disclosure Statement. The authors declare no conflict of interest

Authors contributions.

M. Kolesnyk: management of the research.

V. Driianska: analyzed and interpreted the immunologic data, was a major contributor in writing the manuscript.

N. Stepanova: analyzed and interpreted the data of patients.

O. Lavrenchuk: analyzed and interpreted the immunological data of children.

I. Bagdasarova: analyzed and interpreted the clinical data of children.

T. Poroshina: performed the immunological examination (SLPI, NGAL) in the blood samples.

V. Holod: performed the immunological examination (cytokines) in the blood samples.

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