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# ASSESSMENT OF INTERLEUKIN 17A AND 23 IN THE COURSE OF BLADDER CANCER AND SELECTED BENIGN UROLOGICAL DISEASES

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Several cytokines have been indicated to be significantly involved in urological diseases. Interleukin 17A (IL-17A) and interleukin 23 (IL-23) have recently received attention for their involvement in inflammatory diseases and cancers. The aim of the study was to show changes in the level of pro-inflammatory interleukins IL-17A and IL-23 in patients with bladder cancer (BC) and selected urological diseases. An important cognitive aspect was to study the interdependencies between the studied interleukins and to assess their diagnostic value for such diseases. The material for the study was urine sample from patients with BC, urinary tract infection (UTI), urolithiasis, benign prostatic hyperplasia (BPH), US (urethral stricture), which was compared to the urine sample of healthy people without urological disorders. Interleukin concentrations were measured by the immunoenzymatic method. The levels of IL-17A and IL-23 in the urine of patients with BC, UTI, BPH and US were significantly higher compared to the control group. Statistically significant differences were found in the level of both interleukins compared to the control group in all diseases except urolithiasis. IL-17A and IL-23 correlated with each other in patients with all urological diseases except urolithiasis. The results of the conducted studies showed that selected urological diseases changed the levels of IL-17A and IL-23 in the urine of patients. The observations made confirmed the participation of these interleukins in the course of the urological diseases, especially in BC, and allowed to classify them as potentially useful parameters for diagnostic purposes.

Key words: *bladder cancer, urolithiasis, benign prostatic hyperplasia, urethral stricture, urological diseases, interleukin-17A, interleukin-23, urine, T lymphocytes, inflammation*

## INTRODUCTION

The key role of inflammatory mediators is played by cytokines, hormone-like peptides, mainly produced by immune cells activated by an inflammatory factor. Cytokines, by regulating the proliferation, differentiation and activation of B and T lymphocytes, natural killer cells, monocytes and granulocytes, influence all stages of the immune response. They also interact with each other, inhibiting or stimulating other cytokines (1, 2). Inflammation often accompanies various diseases and plays a role in their initiation and/or course. A number of cytokines have been shown to contribute significantly to urological diseases (3). Interleukin 17 (IL-17) and interleukin 23 (IL-23) have attracted attention over the past decade due to their involvement in inflammatory diseases and cancers (4).

IL-17 is a cytokine newly shown to be involved in inflammation and host defense against infection and adaptive immune systems (5, 6). IL-17 can induce the expression of various proinflammatory factors such as cytokines, chemokines, and MMPs (matrix metalloproteinases) (7). Studies have demonstrated that IL-17 plays a role in tumorigenesis in inflammation-associated cancer, possibly *via* a mechanism mediated by vascular endothelial growth factor (VEGF) and transforming growth factor β1(TGF-β) (8, 9). I L-17 has six family members (IL-17A to IL-17F). Interleukin 17A (IL-17A) belongs to the T helper 17 (Th17) cell subset. IL-17A participates in the allergic and autoimmune response, the process of carcinogenesis and protects against bacterial and fungal infections. IL-17A activates immune cells such as T cells, B cells, and macrophages to promote T cell priming and proinflammatory cytokine production, and nonimmune cells to induce many proinflammatory mediators such as cytokines, chemokines, MMP, VEGF, receptor activator of nuclear factor kappa-B ligand (RANKL) and antimicrobial peptides. These mediators induce neutrophil recruitment at inflammatory sites, promote local tissue destruction, induce neovascularization in tumors, enhance osteoclastogenesis, and protect from pathogens, resulting in disease development and host protection, and so IL-17A seems to be an important player in different diseases (5, 6).

IL-23 belongs to the IL-12 family of cytokines, which in turn is part of the IL-6 superfamily. The main sources of IL-23 are several types of antigen-presenting cells, such as activated dendritic cells, and monocytes and macrophages upon exposure to pathogens. Epithelial cells, including keratinocytes, intestinal

epithelium, and glomerular podocytes, have also been shown to contribute to the production of IL-23 (10, 11). IL-23 plays an important role in both innate and adaptive immunity. It is essential for the coordination of early local immune responses. IL-23 has been shown to induce the production of interferon gamma (IFN-γ), which is very important in Th1 cell response and cellular immunity against intracellular pathogens (12). Moreover, IL-23 plays a leading role in NK cell activation, induction of T cell proliferation and regulation of antibody levels. IL-23 indirectly affects the production of inflammatory mediators by macrophages and dendritic cells (including TNF-α and IL-1β). Functionally, IL-23 has been classified as a pro-inflammatory cytokine, an essential factor in the development of T cell-mediated inflammation. It is a key participant in the central regulation of cellular mechanisms involved in inflammation. There is ample evidence that increased levels of IL-23 are associated with the occurrence of autoimmune diseases (13). The key role of IL-23 in the pathogenesis of inflammatory diseases is related to the Th17 cell line. The initial differentiation of virgin T lymphocytes into Th17 cells requires the presence of factors such as TGF-β, IL-6 and IL-1β, while IL-23 is needed to activate and maintain appropriate levels of Th17. Consequently, interleukin 23 induces Th17 lymphocyte secretion of their major effector molecule, IL-17A. Research shows the participation of interleukin 23 in rheumatoid arthritis, Crohn's disease and other inflammatory bowel diseases, psoriasis and multiple sclerosis (14-17).

IL-17 and IL-23 have also been found to be over-expressed in different human cancers (18). IL-17 induce the proliferation of cervical cancer cells (19). Higher expression of IL-17 in gastric and ovarian cancer have been observed (20, 21). In contrast, several studies have mentioned the antitumor effect of IL-17 as it has been revealed that this cytokine can eradicate tumor cells (22). Besides, IL-17 can induce CD8+ cytotoxic T lymphocyte against melanoma (23). In a cohort of primary non-small cell lung cancer (NSCLC) tumors, IL-23 expression was significantly elevated in patient tumor samples (24). The serum level of IL-23 was significantly elevated in lung cancer patients while the serum's IL-17 level was lower in such patients, but the difference was not statistically significant. IL-17 and IL-23 values were correlated with inflammatory markers in the patients. Presence of comorbid disease (*diabetes mellitus*, hypertension or chronic obstructive lung disease) did not have any effect on the levels of IL-17 or IL-23 (25). Little is known about specific IL-23 alterations associated with breast cancer, and the data available are still controversial. Gangemi *et al.* (26) were first to study the role of IL-23 in breast cancer patients, showing a significant increase respect the control group. The serum levels of Th17 cell-associated cytokines, IL-17 and IL-23 in pancreatic patients were significantly higher than among healthy volunteers. Moreover, levels of IL-17 and IL-23 were significantly higher in stage III-IV tumors than stage I-II tumors (27). The mRNA expression levels of Th17-related factors (IL-17, IL-23p19) in tumor tissues and the serum concentrations of IL-17 and IL-23 cytokines were significantly increased in patients with advanced gastric cancer (7). There is little research on IL-23, and especially IL-17A, in urothelial tumors, which is why they became the object of our research.

The aim of the study was to examine and demonstrate changes in the levels of pro-inflammatory interleukins IL-17A and IL-23 in the urine of patients with urological diseases such as: bladder cancer (BC), benign prostatic hyperplasia (BPH), urinary tract inflammation (UTI), urolithiasis and urethral stricture (US), compared to urologically healthy people. An important cognitive aspect was the study of the interdependencies between the studied proteins and the assessment of their diagnostic value in the presented diseases. No information similar to the conducted research was found in the available literature, which makes the submitted work innovative.

## MATERIALS AND METHODS

### *Patient selection and collection of data*

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Wroclaw Medical University (KB-292/2-16). All participants were informed of the aim of the study and gave written consent to participate.

The study groups consisted of patients with selected urinary diseases: BC, UTI caused by UPEC, urolithiasis, BPH and US treated at the Urology and Oncological Urology Clinic (Wroclaw Medical University).

Before measuring IL-17A and IL-23, patients with BC, BPH, urolithiasis and US whose biochemical tests and microscopic assessment of urine sediment showed the presence of infection or inflammation were removed. This resulted in homogeneous groups of patients.

The control groups (C1 and C2) were selected from participants with no history of cancer or other chronic urological disorders, which was excluded by clinical examination of the cytology of urine sediment and a urine strip test.

Since the group of patients with BC, UTI and urolithiasis consisted of both male and female, comparative analyzes of IL-17A and IL-23 results were performed in comparison to the C1 group (male and female). The group of patients with BPH and US consisted only of male patients, therefore the results in this group were analyzed compared to the C2 (male) group.

### *Clinical classification of patients with bladder cancer*

Patients with BC were grouped according to the stage of cancer: LG (low malignancy) and HG (high malignancy) according to WHO/International Society of Urological Pathology - ISUP 2004 system and depending on the invasiveness of the cancer: non-invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) according to the TNM classification by the Union for International Cancer Control (UICC) in 2009.

### *Experimental procedures*

The research material was the urine of patients and a healthy control group. The urine samples were collected in polystyrene containers (Aptaca, Canelli, Italy), then centrifuged by a MPW-350 laboratory centrifuge (MPW Instruments, Warsaw, Poland) for 10 minutes (1500 rpm), after which the obtained supernatant was removed, placed in microcentrifuge tubes and stored at –80°C for further investigation.

Concentrations of interleukins were measured in urine by the immunoenzymatic method (ELISA) with Enzyme-Linked Immunosorbent Assay Kits: Nori Human IL-17A ELISA Kit-Data Sheet and Nori Human IL-23 ELISA Kit-Data Sheet (Genorise Scientific, Berwyn, PA, USA), according to the manufacturer's instructions in a listed test.

The assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin streptavidin chemistry to improve the performance and sensitivity of the assays. An antibody specific for human IL-17A/IL-23 was pre-coated onto a microplate. Standards (100 µL) and samples (100 µL) were pipetted into the wells and any IL-17A/IL-23 present was bound by the immobilized antibody. After washing away any unbound

substances, a detection antibody specific for human IL-17A/IL-23 was added to the wells. Following a wash to remove any unbound antibody reagent, a detection reagent was added. After intensive wash a substrate solution was added to the wells, and color developed in proportion to the amount of IL-17A/IL-23 bound in the initial step. The reaction was stopped by adding a stop buffer. Absorbance was read at 450 nm with a microplate reader (Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments, Winuski, VT, Germany).

Concentrations of IL-17A and IL-23 in urine were calculated in relation to urine creatinine concentration estimated by Jaffe's routine method based on the reaction of picric acid (Picric Acid, Saint Louis, MO, USA, Sigma).

The determined values of the concentration of ILs in the urine were converted to the concentration of urinary creatinine in order to eliminate the influence of dilution or concentration. Results were reported in pg/mg creatinine for IL-17A and IL-23 in urine.

*Table 1.* Demographic and clinical data of BC patients, patients with benign urological diseases (UTI, urolithiasis, BPH, US) and control groups.

| **Population characteristic** | **Patients Groups** | **Controls** |
|---|---|---|
| | BC | **C1** |
| **N** | 88 | 32 |
| **Age**, y, (range) | 67(85–51) | 63 (40–78) |
| **Man** (N) | 75 | 27 (**C2**) |
| **Age**, y, (range) | 68 (36–85) | 64 (45–82) |
| **Women** (N) | 13 | 5 |
| **Age**, y, (range) | 68 (61–85) | 59 (42–71) |
| | **BC subgroups (N)** | |
| **LG** | 39 | |
| **HG** | 49 | |
| **NMIBC** | 68 | |
| **MIBC** | 20 | |
| | **UTI** | |
| **N** | 17 | |
| **Man** (N) | 12 | |
| **Age**, y, (range) | 62 (33–73) | |
| **Women** (N) | 5 | |
| **Age**, y, (range) | 64 (50–73) | |
| | **Urolithiasis** | |
| **N** | 7 | |
| **Man** (N) | 4 | |
| **Age**, y, (range) | 60 (48–67) | |
| **Women** (N) | 3 | |
| **Age**, y, (range) | 47 (25–73) | |
| | **BPH** | |
| **N** (Men) | 13 | |
| **Age**, y, (range) | 69 (55 – 83) | |
| | **US** | |
| **N** (Men) | 7 | |
| **Age**, y, (range) | 53 (21 – 67) | |

BC, bladder cancer; BPH, benign prostatic hyperplasia; C1, control group (men and women); C2, control group (only men); HG, high grade; LG, low grade; MIBC, muscle invasive bladder cancer; N, number of patients and controls; NMIBC, non-muscle invasive bladder cancer; US, urethral stricture; UTI, urinary tract infection; y, years.

*Statistical analysis*

Statistical analysis was conducted with Statistica PL software (version 13.3). The normality of distribution was checked by Lilliefors and Kolmogorov-Smirnov tests. The nonparametric U Mann-Whitney test was used for the comparison of the variables between groups. The comparison of results within subgroups was performed using the Wilcoxon test. The associations between continuous variables were analyzed by the Spearman test. The receiver operating characteristic curve (ROC) was estimated. The area under the curve (AUC) and cut-off point were calculated. Diagnostic value indicators with 95% CI, such as sensitivity and specificity, were calculated. In all analyses $p<0.05$ was accepted as a significant value.

## RESULTS

*Patient and control characteristics*

One hundred thirty two patients participated in the study, 67% of whom were patients with BC. The remaining patients were: 13% with UTI, 10% with BPH and 5% each with US and urolithiasis.

The control group C1 consisted of 32 people (n=27 male and n=5 female) and the control group C2 consisted of only men (n=27). In terms of gender and age, the study group did not differ from the control group ($p>0.05$). The characteristics of the patients and the control group are presented in *Table 1*.

Median levels and interquartile ranges of urinary IL-17A and IL-23 of patients with BC, subgroups of BC (LG, HG, NIMBC, MIBC), UTI, urolithiasis, BPH, US and control groups with statistical analysis are presented in *Table 2* and *Fig. 1*.

As there were both male and female patients with BC, UTI, and urolithiasis, the ILs results were related to C1. The ILs results obtained from BPH and US (only male) patients were compared to C2.

The median levels of IL-17A in the group of BC patients and subgroups LG, HG, NIMBC and MIBC were 2.5, 2.3, 2.8, 2.5, 2.5-fold higher, respectively, compared to the control group (C1), and there were statistically significant differences ($p\leq0.001$) between these groups. The statistical analysis showed no significant differences between levels of IL-17A in subgroups of BC patients (*Table 2*).

The median levels of IL-23 in group of BC patients and subgroups LG, HG, NIMBC and MIBC were 1.7, 1.5, 1.8, 1.6, 1.7-fold higher, respectively, compared to the control group (C1), and there were statistically significant differences, except for subgroup LG. The statistical analysis showed no significant differences between levels of IL-23 in subgroups of BC patients (*Table 2*).

The highest median level for IL-17A and IL-23 was found in the subgroup of patients with highly invasive BC (HG).

The highest medians of IL-17A were found in the UTI and US patient groups and were both 1.9-fold higher compared to the control groups, respectively, with statistically significant differences ($p\leq0.001$). The median value of IL-17A in patients with BPH was 1.3-fold higher compared to the control and was statistically significant ($p=0.007$). In patients with urolithiasis, the level of IL-17A did not differ statistically significantly compared to the control (*Table 2*).

The highest medians of IL-23 were observed in the UTI and US patient groups and were 1.8, 2.5-fold higher compared to the control groups, respectively, with statistically significant

*Table 2.* Results of IL-17A and IL-23 in the urine of BC, BC subgroups (LG, HG, NIMBC, MIBC), UTI, urolithiasis, BPH, US and control groups with statistical analysis.

| Groups | **IL–17A** [pg/mg] | | **IL–23** [pg/mg] | |
|---|---|---|---|---|
| | **Me** (IQR) | **p*** | **Me** (IQR) | **p*** |
| **BC** | 7.13 (4.37–9.78) | ≤0.001 | 182.68 (130.51–316.11) | ≤0.001 |
| **LG** | 6.40 (3.40–9.77) | ≤0.001 | 170.95 (115.70–323.96) | NS |
| **HG** | 7.60 (4.80–9.80) | ≤0.001 | 197.19 (161.32–308.26) | ≤0.001 |
| **NIMBC** | 7.01 (4.14–9.83) | ≤0.001 | 179.15 (121.69–325.10) | 0.003 |
| **MIBC** | 7.18 (4.50–8.51) | ≤0.001 | 188.54 (166.04–258.33) | 0.004 |
| **UTI** | 5.49 (3.82–6.94) | ≤0.001 | 239.19 (152.52–344.00) | ≤0.001 |
| **Urolithiasis** | 3.86 (3.12–4.89) | NS | 186.86 (122.68–208.48) | NS |
| **BPH** | 3.65 (3.16–6.10) | 0.007 | 162.17 (117.74–294.74) | 0.004 |
| **US** | 5.14 (4.20–8.63) | ≤0.001 | 280.39 (183.47–512.16) | ≤0.001 |
| **C1** | 2.83 (2.29–3.84) | – | 129.45 (97.15–179.34) | – |
| **C2** | 2.74 (2.11–3.51) | – | 110.42 (83.26–160.84) | – |

BC, bladder cancer; BPH, benign prostatic hyperplasia; C1, control group (men and women); C2, control group (men); HG, high grade; IQR, interquartile ranges; LG, low grade; Me, median; MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; NS, not statistically significant; UIT, urinary tract infection; US, urethral stricture; p - statistical difference between patient groups and control groups (*U Mann-Whitney test); p<0.05 - statistically significant value.

differences (p≤0.001). The median value of IL-23 in patients with BPH was 1.5-fold higher compared to the control and was statistically significant (p=0.004). In patients with urolithiasis, the level of IL-23 did not differ statistically significantly compared to the control (*Table 2*).

The statistical analysis showed a significant difference between the level of IL-17A in patients with BC and patients with BPH (p=0.04) and between patients with BC and patients with urolithiasis (p=0.01). No such difference was observed between IL-17A levels in patients with BC or UTI and patients with US. There was no difference between the level of IL-23 in patients with BC and patients with UTI, BPH, US or urolithiasis. Visualizations of the obtained results of IL-17A in urine of patients are presented in *Fig. 1A* and results of IL-23 in urine of patients are presented in *Fig. 1B*.

*Association of urine IL-17A level with IL-23 in patient groups*

The linear significant dependence between urinary IL-17A and IL-23 levels of BC patients was demonstrated (R=0.84; p=0.000) (*Fig. 2*).

Significant corrections between IL-17A and IL-23 were also shown in patient subgroups: LG (R=0.81; p=0.000), HG (R=0.71; p=0.000), NIMBC (R=0.80; p=0.000), MIBC (R=0.58; p=0.007).

Mutual positive strong correction between IL-17A and IL-23 levels was demonstrated in patients with UTI (R=0.83, p=0.000), BPH (R=0.82, p=0.000) and US (R=0.82, p=0.023). There was no correlation between interleukins in patients with urolithiasis.

*Assessment of the urine diagnostic value of IL-17A and IL-23*

To assess the diagnostic value of IL-17A and IL-23, ROC curves were plotted in the patient groups. Sensitivity, specificity, area under the ROC curve (AUC), and cut-off value for IL-17A and IL-23 in the groups of patients (BC, UTI, urolithiasis, BPH and US) are presented in *Table 3*.

AUC values lie in the range <0; 1>. For good diagnostic tests, AUC <0.8–0.95> is acceptable. A good diagnostic value for IL-17A was shown in the group of patients with BC and US, while IL-23 obtained a good diagnostic value in the group of patients with UTI and US.

## DISCUSSION

In the presented study, the changes of the level of two pro-inflammatory interleukins, IL-17A and IL-23, in the urine of patients with urological diseases BC, UTI, urolithiasis and US were analyzed in relation to healthy people without urological diseases (control group). The choice of urine as the research material in the presented study seems to be appropriate, given that diseases of the urinary system are under assessment and that this biological material is easy to obtain. In the course of the disease process, dysfunction and damage to the urinary tract occurs and, as a consequence, inflammatory proteins are released, in urine, where their concentration is often much higher than in other biological material, *e.g.*, serum.

Bladder cancer is the 10$^{th}$ most common type of cancer worldwide. BC is more common in men than in women, with respective incidence and mortality rates of 9.6 and 3.2 per 100,000 in men (about four times higher than for women around the world). Incidence rates in both sexes are highest in Southern Europe, Western Europe and Northern America (28). The main reasons that may explain the higher incidence of BC in more developed countries are: faster economic development; intensification of BC risk factors, including smoking, poor eating habits and the detrimental effects of environmental pollution; occupational exposure (29, 30). BC etiology is a multi-factorial process influenced by both acquired and inherited variables. Smoking is well-recognized as the main risk-factor for developing BC but long-term-inflammation may have a more closely related role (31-33).

A number of cytokines are known to be associated with BC. Interleukins are released in excess by tumor cells and connective tissue stromal cells as well as stimulated host immune cells in BC patients. Measuring some of them is helpful in diagnosing and monitoring the disease. The interleukins described in BC include interleukins with pro-inflammatory effects such as IL-2,

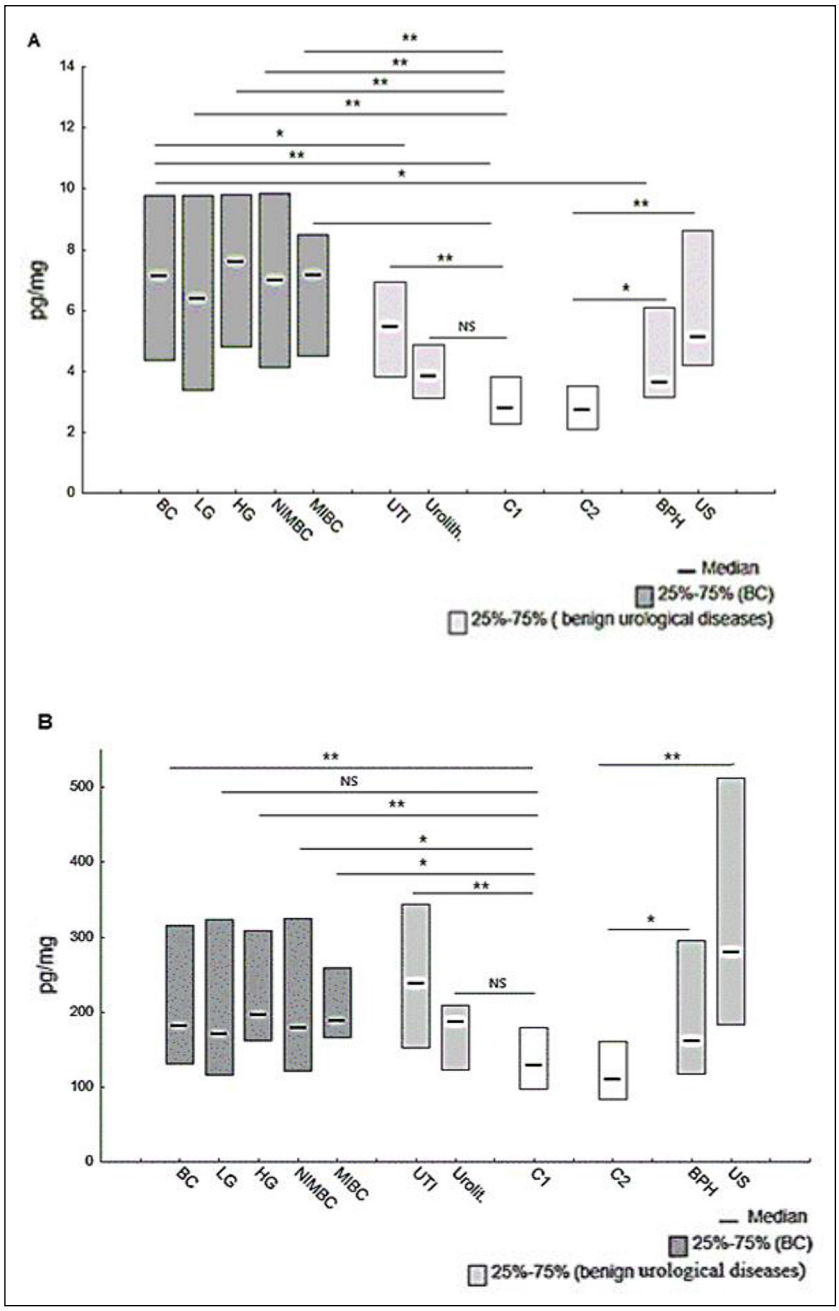


*Fig. 1.* Results of IL-17A (A) and IL-23 (B) in urine of patients and control groups. C1 - control group (man and woman); C2 - control group (only man); statistical difference between patient groups and control groups (U Mann-Whitney test and Wilcoxon test); **p<0.001, *p<0.05. *Abbreviations*: BC, bladder cancer; BPH, benign prostatic hyperplasia; HG, high grade; LG, low grade; MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; NS, not statistically significant; US, urethral stricture; UTI, urinary tract infection.

IL-6, IL-8, IL-18 and anti-inflammatory and immunomodulatory effects such as IL-13 (34).

Urinary tract infections can be defined as microbial infiltration of the urinary tract, which remains sterile in healthy individuals. They are bacterial infections that affect every part of the urinary system, including the kidneys, ureters, bladder, and urethra. Risk factors for developing urinary tract inflammation include female sex, previous episodes of UTIs, sexual activity, vaginal infection, diabetes, obesity, and genetic susceptibility. *Enterobacteria* are one of the most important etiological factors of urinary tract infections, associated with the presence of surface adhesins, which enable proper adhesion of bacteria to the epithelium of the urinary tract. *Escherichia coli* has been documented to be the most common bacterium causing UTIs. Other pathogens, such as *Enterobacter* sp., *Citrobacter* sp., *Serratia* sp., were previously rarely isolated but are now becoming more and more important in the etiology of UTI (35-39).

Infections, especially those caused by highly pathogenic strains of *E. coli*, are often recurrent and dangerous urinary tract infections. Progressing infection may lead to pyelonephritis, acute nephritis and urosepsis. Therefore, the search for specific markers for the early detection of urinary tract inflammation is an important aspect in the prevention and early treatment of these diseases. Markers determined in urine (*e.g.* enzymes, proteins, cytokines) that are directly released from the damaged organ seem to be particularly valuable. An example may be the study by Skowron *et al.* (40) who assessed the usefulness of four markers in urine, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C (CysC), uromodulin (UMOD) and some interleukins (IL-6 and IL-18) in the diagnosis of ascending acute kidney injury (AKI) caused by bacterial pyelonephritis. The study was performed on rats that were inoculated transurethrally with various doses of *Escherichia coli* to induce isolated pyelonephritis. The animals were divided

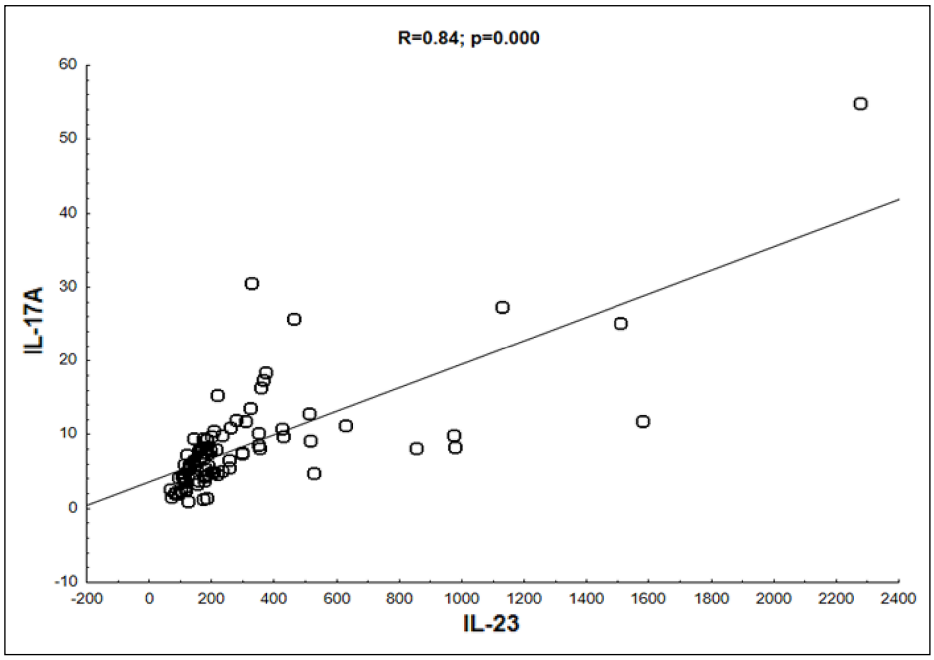


*Fig. 2.* Linear relationships between IL-17A and IL-23 levels of BC-patients.

*Table 3.* Parameters for assessing the diagnostic value of IL-17A and IL-23 in patient groups.

| | **IL-17A** [pg/mg] | | | | **IL-23** [pg/mg] | | | |
|---|---|---|---|---|---|---|---|---|
| | **SE** [%] | **SP** [%] | **AUC** p-value | **Cut-off** [pg/mg] | **SE** [%] | **SP** [%] | **AUC** p-value | **Cut-off** [pg/mg] |
| **BC** | 89 | 98 | 0.83 (p<0.001) | 4.0 | 79 | 80 | 0.72 (p<0.001) | 165.1 |
| **UTI** | 74 | 79 | 0.77 (p<0.001) | 3.7 | 74 | 75 | 0.8 (p<0.001) | 177.1 |
| **BPH** | 73 | 71 | 0.76 (p=0.001) | 3.3 | 85 | 52 | 0.74 (p=0.003) | 112.5 |
| **Urolithiasis** | 88 | 56 | 0.69 (p=0.048) | 3.0 | 75 | 76 | 0.63 (p=0.277) | 152.6 |
| **US** | 100 | 78 | 0.92 (p<0.001) | 3.6 | 86 | 82 | 0.85 (p<0.001) | 183.4 |

AUC, area under the ROC curve; BC, bladder cancer; BPH, benign prostatic hyperplasia; SE, sensitivity; SP, specificity; UIT, urinary tract infection; US - urethral stricture; p<0.05 - statistically significant value.

into three groups to induce isolated pyelonephritis (group 1), AKI due to pyelonephritis (group 2), AKI and urosepsis (group 3). Urine samples were collected before inoculation and 7, 14, and 21 days later. All study groups showed increased uNGAL and uCysC concentrations at all study time points. uKIM-1 concentrations in group 1 were the same as at baseline, while in groups 2 and 3 they were increased at all time points of the study. uUMOD concentrations in groups 1 and 2 tended to decrease with time after vaccination, whereas in group 3 they increased rapidly 21 days after infection. uKIM-1 appears to be the only marker of ascending AKI associated with urinary tract infection. Elevated concentrations of uNGAL, uCysC and uUMOD were found in both AKI and isolated pyelonephritis. This observation seems to have clinical significance and emphasizes the need to search for diagnostic panels composed of different markers.

Recently, it is believed that the altered immunity of the urinary tract is influenced by dysbiosis. Therefore, it is important to examine the local microflora of the affected organs, *i.e.* the urinary bladder microflora in acute/chronic recurrent cystitis or the urinary semen microbiome in chronic prostatitis. It has been shown that the symptoms of chronic prostatitis are related to lower microbial diversity in semen and urine (41).

Chronic inflammation is a risk factor for prostate cancer and benign conditions such as BPH. Epithelial, stromal and inflammatory cells of the prostate produce cytokines (CCL-5, CCL-2), IL (IL-1α, IL-1β, IL-6, IL-18) and hypoxia-inducible factor-1α (HIF-1α), which create a local inflammatory microenvironment. Proinflammatory interleukins are involved in the progression of BPH. IL-17 is involved in the initiation and progression of BPH by activating the nuclear factor-kappa-B (NF-κB) pathway, which leads to the secretion of other proinflammatory cytokines such as IL-1, IL-6, and IL-8 (42, 43).

Urethral stricture is a condition of abnormal narrowing involving a part of the urethra that is surrounded by a spongy body. US is the result of ischemic fibrosis, which manifests as a scar on the spongy body. US has many different etiologies, the four main ones being: iatrogenic, idiopathic, trauma, and inflammation (44).

Our own studies showed statistically significant differences between the levels of IL-17A and IL-23 in patients with BC compared to controls. In the HG, NIMBC and MIBC subgroups, significant differences were also shown between the levels of both interleukins compared to the control, only no significance was found in the level of IL-23 in patients with low invasiveness (LG). The highest levels of both IL-17A and IL-23 were found in patients with high-grade BC (HG). The studies showed significant differences in the urinary levels of IL-17A and IL-23, depending on the invasiveness and malignancy of BC. Strong relationships between IL-17A and IL-23 were observed both in the group of patients with BC and in the subgroups (LG, HG, NIMB, MIBC). The results suggest an increase in the urinary levels of both interleukins with the increase in BC.

Baharlou *et al.* (45) studied the gene expression of IL-6, IL-17, and transforming growth factor beta (TGF-β) in patients with BC. IL-17 expression (as well as IL-6 and TGF-β) was assessed using real-time polymerase chain reaction (qRT-PCR). IL-17 expression was significantly higher in BC patients in relation to healthy people. Since most patients had early stage BC, IL-17, being an important pro-inflammatory cytokine, may play an important role in the recruitment of antitumor immune responses in the initial process of carcinogenesis. IL-17 may also be a predictor in BC prognosis.

Chi *et al.* (46) used flow cytometry to analyze the Th17 cell phenotype in the blood and tumor tissue of BC patients with simultaneous analysis of T(reg) cells. The results showed that Th17 cells were enriched in the tumors of BC patients relative to the peripheral blood of patients and controls. BC patients had a higher percentage of T cells (reg) in peripheral blood compared to controls. Almost all patients showed a relative enrichment in tumor infiltrating T(reg) compared to peripheral blood. The authors suggested that the balance between Th17 and T(reg) cells may play a role in the development or progression of BC.

Wang *et al.* (47) showed that IL-23 expression in non-invasive BC was significantly higher than in muscle-infiltrating BC. IL-23 expression was negatively correlated with BC clinical stage and positively correlated with prognosis. A low concentration of IL-23 promoted T24 cell proliferation, migration, invasion and EMT transformation, while a high concentration inhibited these functions. These results indicated that IL-23 plays a dual role in the progression of bladder cancer. Low concentrations of IL-23 promote bladder tumor progression, while high concentrations have the opposite effect.

Recent studies suggest a potential impact of Th17 cells in tumor immunology. The implication of Th17 cells in bladder cancer can be judged by the expression of their related cytokines and a key transcription factor, retineic-acid-receptor-related orphan nuclear receptor gamma (RORγt), which helps in the development of Th17 cells (48). Therefore, the expression of Th17-related cytokines, RORγt and distribution of Th17 cells require assessment for the involvement of Th17 in bladder cancer to be understood. A study by Chugh *et al.* (49) showed the frequency of Th17 cells to be significantly higher in patients than controls. Circulating levels of pro-inflammatory cytokines IL-17A, IL-23 and IL-6 were also significantly elevated in patients. The authors suggest a possible involvement of Th17 cells in BC urothelial carcinoma.

El-Gedamy *et al.* (50) assessed the levels of CD14-positive TAMs (tumor-associated macrophages) in relation to BC development. TAMs play an important role in tumor progression and response to immunotherapy. Based on immunophenotypic analysis, the number of TAMs carrying CD14 and IL-23 receptor (IL-23R) fractions was determined in BC patients and healthy controls. Serum cytokines IL-23 and IL-17 were also measured. The authors showed higher levels of CD14+ IL-23+ TAMs in BC patients compared to controls, and this increase was correlated with tumor stage. The authors have suggested that CD14-positive TAMs, suppressing IL-23R, are involved in inflammation-related cancers by upregulating the inflammatory immune axis IL-23/17.

The analysis of IL-17A and IL-23 in the diseases presented in the paper (UTI, US BPH, urolithiasis) showed an increase in the urinary level of both interleukins, especially in UTI and US. The study showed a significant difference between the level of IL-17A in patients with BC, BPH and urolithiasis. No such difference was observed between IL-17A levels in patients with BC, UTI and US. There was no difference between the level of IL-23 in patients with BC and patients with UTI, BPH, US and urolithiasis. Mutual correlation between IL-17A and IL-23 levels was demonstrated in patients with UTI, BPH and US. Similar relationships have not been demonstrated only in urolithiasis, which may suggest the lack, or negligible participation, of the measured interleukins in this disease. UTI, US, BPH and urolithiasis are common diseases of the urinary system. Few in the available literature, studies of interleukins IL-17A and IL-23 related to these urological diseases have usually been performed in animal models or their expression in tissues has been tested by immunohistochemistry.

IL-17 is believed to be involved in the local regulation of inflammatory signals. Steiner *et al.* (51) demonstrated minimal expression of IL-17 mRNA in normal prostate, but increased expression of IL-17 mRNA in 80% of T cells and epithelial cells in benign prostatic hyperplasia.

In stromal cells in benign prostatic hyperplasia, Penna *et al.* (52) showed that interferon-γ and IL-17, produced by activated alloantigen-specific CD4+ T cells, induce the production of both IL-6 (a strong autocrine factor growth) and IL-8 (paracrine inducer of fibroblast growth factor-2), which are key growth factors in epithelial and stromal cells in the prostate. These results were consistent with a possible link between prostate stromal-induced autoimmune T-cell responses and prostate hyperproliferation.

Liu *et al.* (53) assessed the immunoreactivities of IL-17A, E, F and their receptors IL-17RA, IL-17BR and IL-17CR, infiltration of inflammatory cells and changes in structural cells, including endothelial cells, fibroblasts and smooth muscle cells in prostate tissues from patients with prostate cancer and with benign prostatic hyperplasia. Immunological staining showed that expression of immunoreactivity for IL-17A, IL-17RA, IL-17E and IL-17F was significantly increased in BPH and prostate cancer compared to control. Compared to these disease states, it was characterized by reduced immunoreactivity to IL-17BR and a reduced number of CD68 (+) macrophages, fibroblasts and smooth muscle cells, although these trends correlated with disease severity in both cancer and benign glandular hyperplasia. The data showed that IL-17A, acting through IL-17RA, but not IL-17CR, contributes to the pathogenesis of cancer and prostate hypertrophy. In contrast, IL-17E interacting with IL-17BR may exhibit anti-tumor activity.

There is also evidence that the growth and survival of IL-17-producing T cells requires the presence of additional factors, including IL-23 (54). IL-23 is essential for the activation and maintenance of an appropriate level of Th17 lymphocytes. Consequently, IL-23 induces the secretion by Th17 lymphocytes of their major effector molecule, which is IL-17A (55). IL-23 receptor overexpression has also been observed in epithelial and endothelial cells in benign prostatic hyperplasia (56).

Sivick *et al.* (57) investigated the role of IL-17A in the course of UTI using an animal mouse model. A strong expression of IL-17A was observed in splenocytes isolated from mice infected with transurethral UPEC antigens. Expression of IL-17A transcription in bladders of infected mice correlated with innate immune response to UTIs, and γδ-positive T cells appear to be a key source of IL-17A synthesis.

In another study in mice, the severity of urinary tract inflammation, susceptibility to kidney colonization and the immune response to uropathogens were monitored. Adult mice were shown to be better able to prevent disease progression, kidney colonization and bacteremia than young or old mice. These differences were mainly due to different cytokine and chemokine profiles, depending on the age of the mice. Adult mice showed higher levels of IL-23 and CCL5 in urine and lower levels of CXCL1 than young and old animals (58).

IL-23 reactivity has been shown to be important for IL-17A expression by γδ T cells in a T cell receptor-independent manner (59). To see if IL-23R is expressed in the bladder and therefore can mediate IL-17A expression by γδ T cells in response to a UTI, the expression of IL-23R in bladder tissue was quantified by qPCR (also in a mouse model). The results showed that IL-23R is present in bladder tissue and may play a role in the rapid expression of IL-17A in response to a UTI.

In the available literature, one article was found concerning the studied interleukins in urolithiasis. Kusumi *et al.* (60) investigated the level of inflammatory cytokines in the urine of adolescents with urolithiasis and demonstrated a reduced level of IL-17A compared to healthy subjects. The results confirm the negligible role of this interleukin in the course of this disease. No information was found regarding studies on IL-17A and IL-23 in US.

The ROC curve showed the potential diagnostic value for IL-17A in patients with BC and US (AUC=0.82; 0.92, respectively) and for IL-23 in patients with UTI and US (AUC=0.80; 0.85, respectively).

The conducted research shows that IL-17A and IL-23-IL may be an interesting direction for deepening the information on the pathogenesis of urological diseases and potential utility of these interleukins in diagnosis or monitoring of urological diseases. Further studies should be undertaken to assess the diagnostic value of the presented interleukins more precisely. The use of urine as a research material for the evaluation of IL-17A and IL-23 changes in selected urological diseases is investigated for the first time.

The results of the conducted studies showed that selected urological diseases changed the levels of IL-17A and IL-23 in the urine of patients. The observations made confirmed the participation of the studied interleukins in the course of the diseases in question, especially in BC, and allowed to classify them as potentially useful parameters for diagnostic purposes. It is worth noting that the tests are not invasive (measurement in a urine sample), which is extremely important in the case of urological patients often exposed to painful, uncomfortable, and invasive diagnostic methods. It is advisable to extend the research on the presented interleukins on more numerous groups of patients and in other urological diseases. Further work may be the key to a better understanding of the etiology and course of the discussed urological diseases, which are still unclear in many respects.

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