Summary

According to the data published by *European Association of Urology* (EAU) bladder cancer is the seventh most frequently diagnosed type of cancer in men worldwide, simultaneously the tenth in the whole population. The incidence rates are 9,5 for men and 2,4 for women per 100 000 person-years, while the mortality rates are 3,3 vs. 0,86 respectively. The early diagnosis significantly improves the prognosis for patients. Survival rates are less favorable in invasive forms of this cancer. In the European Union countries there are certain a discrepancy in particular registers, concerning incidence of BC and the five-year survival rate indicator. It results from the varying availability of screening tests and clinical procedures, as well as the analysis of the obtained partial data. Despite significant improvements in new imaging methods and surgical procedure techniques for patients with urothelial bladder cancer that have occurred in recent years, there are certain discrepancies in the assessment of prognosis based solely on histopathological examination results. Therefore, it seems important to take up the issue of supplementing the standard diagnostics with a biochemical panel, based on which the strategy of further treatment, optimization of pharmacotherapy or the risk of metastasis in the case of *in situ* cancers could be precisely determined.

Objective: The main objective of the study was to assess the concentration of potential diagnostic profiling biomarkers in serum and urine of patients with superficial urothelial cancer of the urinary bladder: MMP-1, MMP-9, MMP-14, MMP-15 and their inhibitors (TIMPs 1-4) and α2-macroglobulin. Then, the correlation analysis of the tested parameters and indication of the preferred material for diagnostic tests. Obtaining a multifactorial model of biomarkers that complements the standard diagnostic panel in patients with diagnosed bladder cancer.

Material and method: The study was approved by the Bioethics Committee at the Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz on 19.01.2021 (no. KB 9/2021). Qualification for the study as well as collection and preservation of the material (serum and urine) were conducted at J.K. Łukowicz Specialist Hospital in Chojnice; Department of Urology and Urological Oncology in Chojnice in 2021-2022. For the study included patients with bladder cancer diagnosis n=20 (pTa/pT1 HG/LG), in which material for analysis was collected twice – blood in a volume of about 10 ml and urine maximum 100ml (total 80 samples), before and within three months after electroresection of the tumor

(examination no. I and II). The control group consisted of healthy volunteers without diagnosed cancer.

Statistical analysis: Analysis of the data was performed by using Statistica 13.3 software. The results were presented as mean value with standard error and median. The conformity of the distribution of blood and urine test results before and after tumor resection with the normal distribution was estimated using the Shapiro-Wilk test. Statistical significance was assessed using the non-parametric Wilcoxon signed-rank test. The significance level was set at $p < 0.05$.

Results: The concentration of analyzed markers in the serum of patients with bladder cancer did not differ and were similar to control values, except for MMP-1. In the tested urine samples, the concentration of MMP-1, MMP-9 and MMP-15 did not differ compared to the control group; however MMP-14 was significantly higher in the group of healthy volunteers. **The increase in concentration after tumor resection in the serum of patients with BC was observed only in the case of** MMP-1. In the urine samples significant increase of MMP-9, MMP-14, MMP-15 as well as TIMP-3 and TIMP-4 after surgery intervention was observed.

Conclusions: Evaluation of the concentration of MMPs and TIMPs in the serum of patients with urothelial bladder cancer, does not meet the characteristics of a potential tool supporting the diagnosis of patients. The level of α2-makroglobulin in blood and urine does not reflect the changes that occur within the bladder tumor and its environment. As a supplement of standard diagnostics, assessment of changes in concentration of a panel of metalloproteinases and their inhibitors in urine should be considered. However, assessment the level of only one type of endopeptidase or TIMP has poor clinical value. It has been shown that the size of the tumor does not impact for the levels of particular MMPs and TIMPs in urine. Correlation analysis of urinary MMPs and TIMPs concentration, both before and after electroresection of tumor, may be a helpful tool for biochemical diagnosis. Particularly: MMP-15/TIMP-3; MMP-15/TIMP-4 and MMP-9/TIMP-1.

Keywords: metalloproteinases, MMPs, TIMPs, α2-macroglobulin, bladder cancer