Summary

Renal cell carcinoma (RCC) is the third most common urological malignancy, in which about 30% are diagnosed with a metastatic disease at the time of diagnosis. Due to radiation resistance and chemoresistance, the prognosis of these patients is poor. Therefore, the optimal treatment is complete removal of the tumor from at the stage of localized disease.

The prognosis of patients has improved in recent decades due to an increase in the number of incidentally detected kidney tumors and also the introduction of new types of treatments. Given the general trend of increasing randomly diagnosed kidney tumors, we were inspired to evaluate patients operated for kidney cancer with respect to the type of disease detection and the method of primary examination.

The fact that we do not have a biomarker corresponding to the treatment or recurrence of the disease for monitoring patients after kidney cancer surgery led us to the CTC investigation. CTC examination belongs to the group of liquid biopsy tests and one of its main advantages is the relatively minimal invasiveness (blood collection), which really allows monitoring of tumor dynamics. The primary question was whether CTC cells could play a role in monitoring patients after surgery and event. In the setting of immunotherapeutic and antiangiogenic treatment thanks to molecular CTC analysis at the level of gene expression.

The epidemiological study from May 2011 to May 2016 included 471 patients who underwent surgery for RCC. Data collection took place retrospectively. The reason for the examination according to clinical difficulties and the type of initial examination (US, CT, MRI) were evaluated. Among those examined were 304 (64.5%) men. The mean age of the patients was 64 years (95% CI: 63-65) years. The mean BMI was 28.5 (95% CI: 28-29).

In the monitored group, we observed 347 (73.7%) cases of incidental, 77 (16.3%) symptomatic. In 47 patients (9.9%) it was not possible to distinguish the type of capture.

The distribution of individual stages of detected tumors is the same in men and women. The incidence of early and advanced stages was not dependent on gender, BMI or antihypertensive therapy. The lower stage of the disease was significantly more

diagnosed with ultrasound. Histologically, clear cell RCC (80%) dominated, followed by papillary renal RCC (10%). The incidence of individual cancers was not dependent on early and late stage

As part of CTC testing, peripheral blood (2x8 ml EDTA) was collected from all patients at several time points (1. before surgery, 2. 4-10 days after surgery, 3. approx. 6 weeks after surgery and 4. at 6-12 months after surgery. operations). A total of 495 CTC tests were collected.). A cell size separation protocol (MetaCell®) was used to enrich CTC. After simple filtration of the blood through a porous membrane, the captured cells (greater than 8 μm) are incubated in vitro for a short time (3-5 days). After incubation, cells are stained with vital fluorescent dyes (NucBlue®, Celltracker®). The presence of CTC is evaluated in two steps: first by cellular cytomorphology, second by molecular testing (qPCR analysis). Gene expression analysis of the following genes (ACTB, MUC1, KRT18, KRT19, VIM, CD24, CD44, CD68, CD45, PD-L1 (CD274), VEFG, VEGFR (FLT1), HIF1A, WT) was performed in the fraction of captured cells and was comparison was made with the white blood cell fraction for each patient at different sampling times.

In our study, we demonstrated the presence of CTC in up to 86.7% of tested blood samples from patients operated for kidney cancer. The likelihood of detecting CTC increased with increasing tumor size, especially in clear cell RCC. Gene expression analysis revealed increased gene expression in enriched CTCs for the KRT18 and vimentin genes compared to the white blood cell fraction. It also turned out that the nature of CTC changes during the period under review. From a therapeutic point of view, the most significant changes observed in patients were increased VEGF (vascular endothelial growth factor and / or PD-L1 (Programmed death ligand 1, CD274) expression on CTC.