

## **10. Streszczenie rozprawy doktorskiej w języku angielskim**

The doctoral dissertation comprises 6 articles published in impact factor (IF) journals. The presented works, which I am the author and co-author, were created thanks to my efforts and initiative based on the resolution of the OIL Bioethics Committee, which positively assessed the research project entitled: "Assessment of the usefulness of microRNA as a biomarker in diagnostics and prognosis in patients with prostate cancer". At the same time, I was appointed the coordinator of these studies.

The significance of prostate cancer, both in terms of enhanced detection and life extension, has increased dramatically.

The current diagnostic methods are imprecise both in respect of diagnosing the cancer itself and determining its nature, i.e. malignance and stage. This has a material bearing on further course of action with the patient, particularly concerning treatment methods.

Therefore, we are in urgent need of developing new, non-invasive cancer markers enabling early detection of aggressive cancers.

The primary achievement of the dissertation is having demonstrated that application of various diagnostic biomarkers, such as:

- multiparametric magnetic resonance imaging (mpMRI) (radiological biomarkers),
- SelectMDx genetic test (genetic biomarkers),
- microRNA (miRNA) molecules (molecular biomarkers),

has enhanced the efficiency of the diagnostic process consisting in qualifying patients for prostate biopsy.

The objective of study no. 1 (as listed) was to analyze serum miRNAs as non-invasive biomarkers in patients diagnosed with prostate cancer and to compare it to that of healthy individuals. This preliminary study included a cohort of 62 patients aged, on average, 68.6 with the average PSA of 21.3 ng/ml. Eight healthy men constituted the control group. The miRNA level was

determined quantitatively from the total RNA fraction extracted from serum, marking five miRNA levels (miR-106b, miR-141, miR-21, miR-34a and miR-375) quantitatively using RT-qPCR. The miRNA types were selected based on the data from the writings analyzed. In the analyses, the correlation between clinical and pathological data and the miRNA expression levels was assessed. The relative expression coefficients of miR-106b, miR-141-3p, miR-21 and miR-375 were significantly increased (1.8- 1.9-, 2.4 and 2.6-times, respectively) within the group diagnosed with prostate cancer in comparison with the healthy control group. The highest area under the receiver operating characteristics curve (ROC), that of 0.906, was obtained for miR-357, which indicates excellent diagnostic properties of this biomarker. An expression level of miR-34a, unrelated to cancer, was identified. The scores corroborate earlier data found in the writings, pertaining to potentially identifying prostate cancer patients from among healthy populations by marking appropriate miRNAs (miR-141-3p, miR-21 and miR-375).

Study no. 2 analyzed 40 patients using the following data: prostate specific antigen (PSA), mpMRI test results and Gleason score (based on prostate biopsy) - 8 of the patients (20%) were diagnosed with cancer. The mpMRI was conducted at the very beginning of the diagnostic path in order to ascertain whether prostate biopsy may be potentially required.

The Spearman coefficient was used to assess the correlations between the characteristics at hand. The diagnostic efficacy was assessed as the area under the curve (AUC) of the ROC curve characteristics analysis. Within the entire cohort, 55% of the patients underwent a primary biopsy, while 45% of them - suspected of prostate cancer - had a repeat prostate biopsy. Forty alarming lesions were identified on MRI images using PI-RADS 1 - 5%, PI-RADS 2 - 17.5%, PI-RADS 3 - 32.5%, PI-RADS 4 - 27.5% and PI-RADS 5 - 17.5%. The highest correlation was observed for the mpMRI results and the Gleason score with the Spearman coefficient of 0.41 (95% CI: 0.104-0.646). It follows from

the ROC analysis that mpMRI differentiates between referring patients for prostate biopsy or active supervision with AUC = 0.771 (0.117, 95% CI: 0.542-1.001).

The subject matter of study no. 3 was the bearing the SelectMDX genetic test may have on the prostate cancer diagnostic process. The test is based on isolating the mRNA biomarker in urine. The presence of certain HOXC6 and DLX1 genes mRNA levels is ascertained in order to estimate the risk of both: prostate cancer during biopsy and high-risk prostate cancer (Gleason score  $\geq 7$ ). The prostate biopsies in patients with positive SelectMDx test results confirmed the prostate cancer diagnosis and Gleason score  $\geq 7$ . These patients were subject to radical treatment. The patients with negative test results (low risk) avoided undergoing prostate biopsy forthwith and are under further routine observation and supervision.

The objective of study no. 4 was to assess the possibility of using miRNA molecules to stratify prostate cancer and benign prostatic hyperplasia (BPH) patients. Nine miRNA molecules (-21, -27b, -93, -141, -205, -221, -182, -375 and let-7a) with the highest differentiating potential reported in the writings were used in comparative studies of serum samples and prostate tissue. The Spearman correlation coefficient and the ROC analysis were applied in order to ascertain the capacity of serum miRNA to differentiate PCa and BPH patients. This study clearly shows that miR-93 and miR-375 may be considered as single, non-invasive blood molecules in order to differentiate between PCa and BPH patients. We indicate that two miRNAs have six common target genes correlated with PCa (CCND2, MAP3K2, MXI1, PAFAH1B1, YOD1, ZFYVE26), which have a common molecular function of binding proteins (gene ontology (GO) term: 0005515). High diagnostic value of new serum miR-182 was also described (AUC = 0.881, 95% confidence interval, CI = 0.816-0.946,  $p < 0.0001$ , sensitivity and specificity 85% and 79% respectively).

Study no. 5 is an overview describing the currently recommended prostate cancer diagnostic methods, as well as other tests and biomarkers applied in screening tests. To that end, scholarly publications were analyzed, Scientific Associations' guidelines and expert assessments were reviewed. The study discusses the latest methods recommended for PCa diagnostics, such as: PSA (prostate specific antigen), PHI test, 4Kscore test and other new diagnostic technologies, among them Progenesa, SelectMDx, OncotypeDx and ExoDx Prostate IntelliScore tests.

Study no. 6 summarizes the current body of knowledge about the role of miRNA molecules in prostate cancer diagnostics. They are a class of small, approximately 22 nucleotide-long, non-coding RNA molecules which participate in post-transcription expression regulation of a number of genes. This review describes the significance of miRNA in androgen receptor signalling (AR), cell cycle, epithelial-to-mesenchymal transition (EMT), cancer stem cells (CSC) transformation, as well as its role as a prostate cancer therapeutic tool. The most promising group are the molecular markers, among which much is expected of the use of extracellular miRNA molecules. Modern oncological practice is very much in need of identifying better, miRNA-based, PCa biomarkers replacing current PSA measurements.

### **Doctoral dissertation findings and conclusions**

In the course of the studies conducted, the highest correlation was observed for the mpMRI results and the Gleason score with the Spearman coefficient of 0.41 (95% CI: 0.104-0.646). It follows from the ROC analysis that mpMRI differentiates between referring patients for prostate biopsy or active supervision with AUC = 0.771 (0.117, 95% CI: 0.542-1.001). In general terms, the studies conducted corroborate data presented in the writings and pertaining to successful application of mpMRI - prior to biopsy - to the modern PCa diagnostic path. Further, efficiency of per rectum biopsy is increasing due to enhanced detection

of prostate cancer during mpMRI data guided biopsy. Owing to mpMRI, non-csPCa patients managed to avoid undergoing biopsy. The results and conclusions obtained are similar to those presented in the international PRECISION study, which showed the benefits of mpMRI at the very beginning of the diagnostic path in patients potentially suffering from prostate cancer.

Prostate biopsies in patients with positive SelectMDx test results confirmed the prostate cancer diagnosis and Gleason score  $\geq 7$ . These patients were subject to radical treatment. The patients with negative test results (low risk), in turn, avoided undergoing prostate biopsy forthwith and are under further routine observation and supervision.

In the analyses of miRNA molecules, the correlation between clinical and pathological data and the selected miRNA expression levels was assessed. The relative expression coefficients of miR-106b, miR-141-3p, miR-21 and miR-375 were significantly increased (1.8-, 1.9-, 2.4 and 2.6-times, respectively) within the PCa patients group in comparison with the control group of healthy individuals. The highest area under the receiver operating characteristics curve (ROC), that of 0.906, was obtained for miR-375, which indicates excellent diagnostic properties of this biomarker. The studies findings corroborate earlier determinations, pertaining to potentially identifying prostate cancer patients from among healthy populations by marking appropriate miRNA (miR-141-3p, miR-21 and miR-375).

Summarizing, one ought to propose that due to the heterogeneous nature of prostate cancer, one diagnostic test will not answer all of the questions posed, therefore applying several diagnostic methods will enable physicians to better personalize clinical counsel for patients.