Summary

Prostate Cancer is the second most common type of cancer among men nowadays. One of the biggest challenges to overcome is proper diagnosis and risk stratification, as currently used PSA-level-based methods do not provide satisfactory results for Active Surveillance (AS) approach decision making. Extracellular Vesicles (EVs) are membranous nano-sized vesicles released by all types of cells. One of their most interesting features is that they can carry various classes of molecules (proteins, DNAs, RNAs, metabolites, lipids). As it was proven that EVs cargo might be successfully analyzed despite their nano-sized nature, they are a potentially interesting material for diagnostical procedures of Prostate Cancer.

The aim of this study was an analysis of the small EVs purification method from the serum and urine of the prostate cancer patients for further investigation of the potential molecules that might serve as biomarkers. In addition, another aim was an analysis of the surface markers and miRNA profile of the medium-sized plasma EVs (mEVs) for the potential of distinction between patients classified for AS and not classified for AS, because of aggressive cancer characteristics, based on the histopathological Gleason Score results.

The study included 39 Prostate Cancer patients. Among those, 15 patients were recruited for the comparison of sEVs purification methods and 24 for analysis of medium-sized plasma EVs (mEVs) diagnostic potential for AS risk stratification. For the comparison of sEVs purification methods: precipitation, size exclusion chromatography (SEC) and immunomagnetic separations (ImSep) were chosen. For the analysis of the diagnostic potential of mEVs for AS risk stratification, surface markers: CD9, CD81, PSMA and EpCam, were analyzed with nanoFlow Cytometry, and the miRNA profile of mEVs was checked.

The comparison of the purification protocols for sEVs from serum revealed that the precipitation method significantly affects the size of the obtained sEVs. Moreover, samples obtained from serum by precipitation provided much higher protein contamination than other approaches. In the case of urine, no significant differences were found besides the lower number of CD9-positive sEVs from the ImSep method. The analysis of plasma mEVs surface markers revealed that a ratio of PSMA+ EVs to PSMA+CD9+ EVs provided a significant difference ($p<0,001$) between the AS and non-AS patients. Analysis of the miRNA

profile of plasma mEVs revealed that miR-99a-5p ($p<0,01$), miR-125b-5p ($p<0,05$), miR-145-5p ($p<0,05$) and miR-365a-3p ($p<0,01$) level was significantly higher for non-AS classifying prostate cancer patients.

In conclusion peripheral blood serum and urine of prostate cancer patients using proper purification methods can be considered feasible sources of sEVs. Compared sEVs purification methods: Precipitation, SEC, and ImSep differentially affect the sEVs size, protein contamination and tetraspanins presence. SEC presents the best results in the case of protein contamination of obtained sEVs from both peripheral blood serum and urine compared to other methods. The method of sEVs purification should be guided by the biofluid used as a source of sEVs and further planned analyses. Peripheral blood plasma of prostate cancer patients can be a source of PSMA+ mEVs secreted by the prostate gland cells for further studies. nanoFlow Cytometry analysis of peripheral blood plasma mEV surface markers and miRNA profiling provides a new, potentially better non-invasive alternative for PSA measurements to stratify the risk of prostate cancer progression during active surveillance. Among analyzed potential markers PSMA+/PSMA+CD9+ ratio and upregulation of miR-99a-5p, miR-125b-5p, miR-145-5p and miR365a-3p presented the most promising results that need to be confirmed with a bigger group of patients.