PANEL OF URINARY DIAGNOSTIC MARKERS FOR NON-INVASIVE DETECTION OF PRIMARY AND RECURRENT UROTHELIAL URINARY BLADDER CARCINOMA

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Objectives:
To determine the combination of urinary protein markers for non-invasive detection of primary and recurrent urothelial bladder carcinomas.

Patients:
The study included 240 individuals, out of which there were 50 healthy control subjects, 66 patients with a history of NMIBC without current disease and 124 consecutive patients with bladder cancer. In 8 patients, urothelial carcinoma was not histologically confirmed. After the exclusion of 8 more patients with positive urine culture, there were 49 healthy control subjects, 61 patients with a history of NMIBC without current disease and 114 patients with bladder cancer. Out of 114 patients with bladder cancer, 70 patients had primary occurrence of bladder cancer and in 44 patients it was cancer recurrence, where the original histology was urothelial NMIBC.

Urinary biomarker analysis:
Levels of individual markers, which included α-1-antitripsin, adipocyte-type fatty acid-binding protein (AFABP), resistin, clusterin, uromodulin, zinc-alfa2-glycoprotein (ZAG), heat shock protein 27 (HSP27), angiogenin, calreticulin, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), chemokine (C-X-C motif) Ligand 1 (CXCL1), interleukin 13 (IL-13), vascular endothelial growth factor-A (VEGF-A), carbonic anhydrase 9 (CA9), annexin-V, synuclein-gamma, apolipoprotein A-II (ApoA2), neural cell adhesion molecule (NCAM1; CD56), chromogranin A, proepithelin (progranulin), midkine, T-cell immunoglobulin- and mucin-domain-containing molecule (TIM-4), apolipoprotein A-I (ApoA1), galectin-1, cystatin B and heat shock protein 60 (HSP60) were determined by standard commercially available enzyme-linked immunosorbent assay (ELISA) kits in compliance with the protocol and manufacturer's recommendations. Neuron-specific enolase was examined by the electrochemiluminiscence immunoassay (NSE, ECLIA, Modular, Roche).

Data analysis:
The model was selected through multiple regression (General Linear Model), when the presence of a tumor represented the response variable and marker level the predictive variable. Statistically significant factors from multiple regression were consequently verified by non-parametric location tests (Wilcoxon Two-Sample Test). After the selection of a predictive model, non-parametric ROC (receiver operating characteristic) curves were created. Relative potential of the selected combinations of markers to identify bladder carcinoma was specified by the calculation of area under the ROC curve (AUC). Individual AUC values were compared by the chi-square test. Statistical significance was set at $p < 0.05$ and all reported p values were 2-sized.

Comparison of patients with primary occurrence of bladder cancer and healthy control subjects:
BOX PLOT DIAGRAM for diagnostic model, whose part was cytology in combination with markers Midkine and Synuclein G and ROC curve for the combined test cytology + Midkine + Synuclein G, AUC – 0.9485.
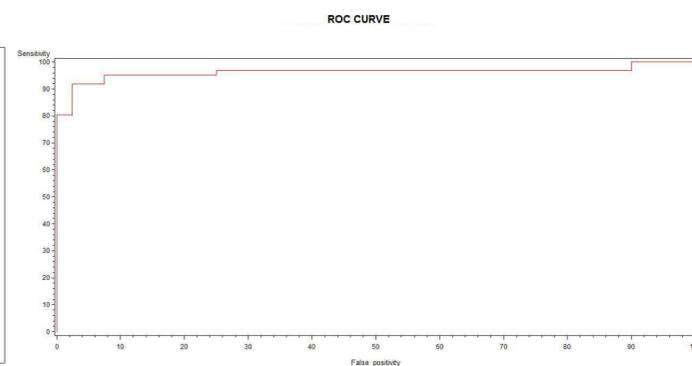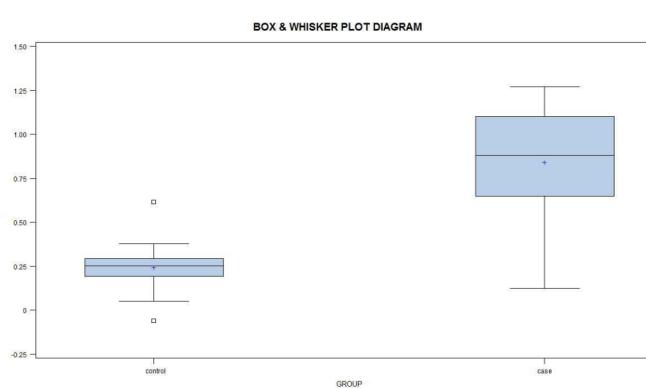
Comparison of patients with bladder cancer recurrence and patients with a history of NMIBC without current disease: BOX PLOT DIAGRAM for diagnostic model which consists of urine cytology and erythrocytes count in urine sediment combined with markers Midkine, Synuclein G, ZAG 2 and CEACAM1 and ROC curve pro combined test cytology + urine sediment erythrocytes count + Midkine + ZAG 2 + CEACAM1 + Synuclein G- *red curve* (AUC – 0.932) and combined test cytology + Midkine + Synuclein G- *green curve* (AUC- 0.885).
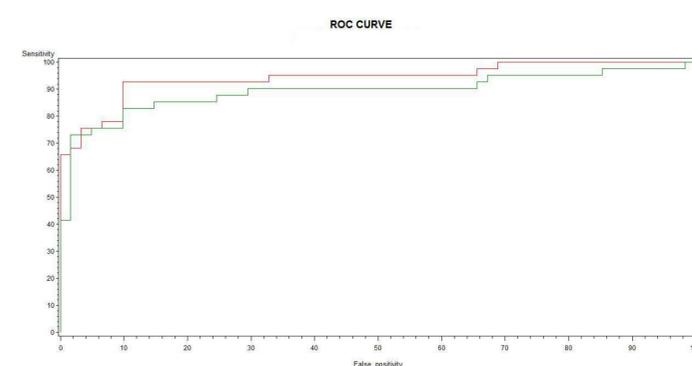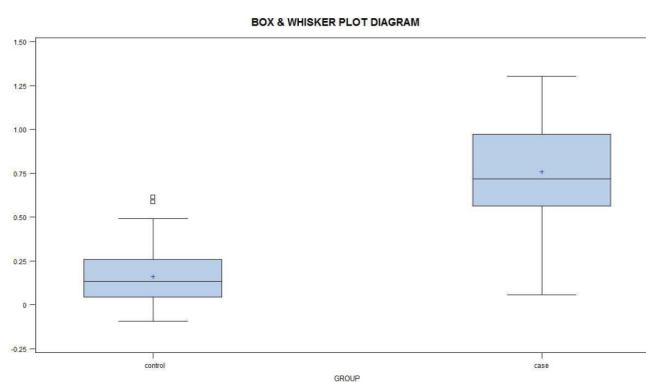
Conclusions:
Our results suggest that the multi-marker test combining cytology and urinary levels of Midkine and Synuclein G can improve the detection of bladder cancer during the primary diagnostics and also during monitoring of patients with NMIBC. To achieve sensitivity and specificity of the combined test over 90 %, more markers have to be studied in followed-up patients than those employed during the detection of bladder carcinoma primary occurrence. To confirm the benefits, it is necessary to carry out other, larger studies.