The usability of cytological and molecular tests in the pre-clinical diagnostics of non-invasive urothelial carcinoma. Case study

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In the presented case, a 66 years old male, neither exposed to harmful occupational factors nor smoking, demonstrated lower urinary tract symptoms (LUTS) without hematuria. In view of the lack of improvement after an initial conservative treatment, an extended diagnostic workup was applied with urethrocystoscopic examination, identifying features of bladder outlet obstructions with no unequivocal macroscopic changes in the bladder mucosa. The patient had for almost two years been pharmacologically treated, without any discernible improvement.

A retrospective analysis of intraoperatively obtained video records triggered the need to apply the following tests: Cytourofish$^{(+)}$ (a cytological study of urinary sediments, together with FISH- 9p21, 9cen, 17cen chromosome probes and IHC *p53*) and the Bladder EpiCheck test of DNA samples, acquired from urinary sediments (15 proprietary methylation biomarkers). The Bladder EpiCheck enabled to determine the EpiScore, which is a measure of the overall biomarker panel methylation level, ranging from 0 to 100. The test cut-off is an EpiScore of 60, meaning that all the results, either equal to or above 60, are considered positive, while those below 60 are considered negative. In the presented case, the EpiScore value was 92 – thus positive. In the course of a subsequent urethrocystoscopic procedure, a randomised biopsy of the bladder mucosa was carried out. A histopathological analysis (endorsed by IHC reactions: *CK20*, *p53*, *Ki67*) of the collected material demonstrated the presence of an urothelial carcinoma in situ. A particular attention was also given to the presence of chromosome 17 polysomy in 100% of the evaluated urinary sediment cells, as well as to the homo-24% and hemizygotic-60% loss in 9p21 sequence. A next-generation sequencing analysis of a DNA sample, acquired from urine sediments, allowed for reproduction of the hypothetical carcinogenesis process activation pathway in the presented case. A coverage analysis of chromosome 9, with the resolution levels of 1 Mb and 15 Mb, revealed deletion of the first 32 Mb of its short arm (9p21-24), confirming the 9p21 deletion, detected originally by Cytourofish$^{(+)}$. The deleted region contained over 300 genes, including *CDKN2A* that showed a hemizygous variant in the 3′ UTR (rs11515). WES detected a probably pathogenic *TP53* variant c.524G>T (p.Arg175Leu) and two, till then, unreported *RB1* missense variants. The results of either test indicated the presence of a urinary tract carcinoma. Those

observations highlighted the role of modern, non-invasive, pre-clinical tests in the diagnostics of this disease. Their early implementation may then be considered legitimate and necessary to confirm/exclude the presence of bladder carcinoma, when it comes to LUTS-like symptoms, which may be concomitant to neoplastic changes.