

**Genomic subtypes of bladder cancer with distinct methylation profiles could be better identified by the Bladder EpiCheck test as compared to cystoscopy/urine cytology**

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**Introduction & Objectives:** Bladder cancer (BC) is a disease with high recurrence rates in treated patients (pts). Surveillance to allow early identifications of recurrence or progression is crucial and based on cystoscopies. Novel non-invasive assays based on genetic profile of cancer cells have been introduced. The Bladder EpiCheck (BEC) analyses DNA from urine of pts and has been shown able to detect subtle disease-specific DNA methylation changes in BC

**Materials & Methods:** Spontaneous urine samples from 88 consecutive pts submitted to cystoscopy for first diagnosis of suspected BC or in follow-up (FU) were collected. In 3 samples we did not find enough DNA for analysis. DNA from the others was extracted and stored. Of these, 77 have been evaluated using the BEC. Genomic DNA was extracted from the cells obtained by urine centrifugation and digested using a methylation-sensitive restriction enzyme. The methylation status of 15 biomarkers was assessed by RT-PCR and analysed. Sample with an EpiScore $\geq$ 60 are considered positive and associated to a high probability of BC. The study was conducted both in the Clinic and in the Laboratory. Physicians and Scientists were not aware of clinical data. Statistical analysis was performed using the IBM SPSS Statistics

**Results:** Final BEC tests were available for 75 pts. In 21 cases it was a first cystoscopy for suspected BC, while 54 pts were in FU (median FU: 3 yrs). 26 pts underwent diagnostic cystoscopies, 45 TUR and 4 RE-TUR for high grade BC. 7 pts presented positive spontaneous urine cytologies and 9 had positive post-cystoscopy wash cytologies. BEC was positive in 23(31%) and negative in 52(69%) pts. All cases of benign lesions, cystitis and 1 case of schistosomiasis (5 cases) had a negative result. Among the 23 positive tests, 14(61%) had a positive pathology for BC: 2 low-grade and 12 high-grade BC; 9 pts with positive BEC, all in FU, did not have a positive pathology of resected tissue but 4 cases had a positive wash cytology (3/4 high-risk BC). Among the remaining 5, 3 had a history of CIS-T1HG BC and 2 with previous intermediate/low risk BC did show macroscopic evidence of disease at cystoscopy, but the TUR pathologies was non diagnostic. 52 pts had a negative BEC result: none had high-grade BC at histology, 5 had low-grade BC and 3 showed suspicious wash cytology (2 with CIS history)

**Conclusions:** The BEC test showed high specificity and 100% NPV in high grade BC. It showed that in positive cases is able to select subsets of BC with negative cystoscopy and positive/negative cytology which seem to present DNA methylation changes. These pts could represent a genetic high-risk population which is pulled out by the BEC and deserves clinical attention. The test could show in negative cases a population of high risk pts with a more stable DNA methylation pattern which could present a inferior risk of progression