

An Innovative Integrative Method for Bladder Cancer Diagnosis

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This commentary refers to the article "Urine DNA methylation assay enables early detection and recurrence monitoring for bladder cancer," by Chen et al. (2020), doi: 10.1172/JCI1139597

Bladder cancer is the most common and lethal cancer of the urinary system in the world [1]. Three-quarters of patients would present with non-muscle-invasive bladder cancer (NMIBC). Although it does not invade the muscle, patients have the risk of recurrence, with a recurrence rate of 70%. Frequent tests for surveillance and repeated treatment for recurrent bladder cancer pose a substantial economic burden [2]. Cystoscopy followed by biopsy is the gold standard for surveillance due to its high sensitivity; however, this approach is costly and invasive [3]. Noninvasive methods by urine testing are attractive alternatives since urine is easy to obtain. Urine cytology detects bladder cancer with high specificity but with low sensitivity. Although UroVysion fluorescence in situ hybridization (FISH) shows an increased sensitivity over urine cytology from 25%–35% to 60%–80%, the sensitivity of FISH is still low and unsatisfactory for patients with low-grade or small tumors [4]. Thus, novel markers are needed to detect and monitor tumor recurrence in bladder cancer patients.

DNA methylation plays a vital role in the epigenetic regulation of tumor-associated genes for decades [5]. Unlike somatic mutation, DNA methylation features tissue specificity and expression consistency. Hypermethylation of tumor suppressor genes occurs in the early stage of tumorigenesis; therefore, it can be detected early [6]. Methylation of CpG sites in urine is a promising marker to detect or monitor bladder cancer [7, 8]. A panel of 15 methylation markers termed Bladder EpiCheck™ was introduced in Europe to monitor NMIBC recurrence [9]. In the multicenter clinical trial, Bladder EpiCheck™ showed a sensitivity of 68.2%

and a specificity of 88.0%, but the sensitivity increased to 91.7% as the low-grade Ta recurrences were excluded. A method based on four methylation markers (VIM, RASSF1A, GDF15, and TMEFF2) were reported to diagnose bladder cancer with a sensitivity of 82%. However, this result is not yet verified, and its specificity of 53% also limited the application [7]. Another multicenter study combined the three-gene methylation (*CFTR*, *SALL3*, and *TWIST1*) results and cytology results. It can identify bladder cancer with a sensitivity of 96% and a specificity of 40% [10]. Up to now, the methylation markers showed great potential to surveil bladder cancer recurrence with good sensitivities, but the specificities are relatively low. Furthermore, DNA methylation detection did not improve the efficacy of identifying low-grade recurrence, warranting new and innovative approaches.

Chen et al. [11] reported a novel method, urine tumor DNA Methylation MassARRAY, termed utMeMA, to detect urine tumor DNA methylation by MassARRAY. They first identified 26 significant methylation markers of bladder cancer in the combined analyses of cohorts from Sun Yat-sen Memorial Hospital (SYSMH), The Cancer Genome Atlas (TCGA), and the Gene Expression Omnibus (GEO) [12] database. A diagnostic model that included only two CpG markers (cg21472506 and cg11437784) was built to distinguish bladder cancer patients' subtypes. This model was trained and tested in the SYSMH cohort of 313 samples and subsequently validated in a multicenter, prospective, independent cohort of 175 samples. The overall sensitivity was 90.0%, and the specificity was 83.1%, which performed better than urine cytology (sensitivity of 58%) and FISH (sensitivity of 68.7%). For NMIBC patients, the utMeMA showed a higher sensitivity of 85.5% in modeling and validation cohorts than 67% of the aforementioned Bladder EpiCheck™ [9].

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utMeMA also showed the potential of overcoming the barrier of detecting early-stage, minimal, residual, and recurrent bladder cancer, which was often missed by cytology and FISH [13]. utMeMA could discriminate Ta and low-grade tumors with a sensitivity of 64.5%, which was significantly improved compared with urine cytology (11.8%), and FISH (15.8%). These results suggest that utMeMA could be applied to the early screening of bladder cancer patients. In detecting minimal tumors, utMeMA had a sensitivity of 93.3%; meanwhile, the sensitivity of cytology and FISH was 14.8% and 37.9%, respectively. The smallest tumor identified by utMeMA was only 4 mm in diameter, which is likely to be missed even by cystoscopy. The utMeMA may cover the shortage of the difficulty to identify small size tumors in clinical practice. It is recommended that high-risk patients with non-muscle invasive tumors perform repeated transurethral resection of bladder tumor (Re-TURBT) according to the European Association of Urology guideline. There is a lack of approach to evaluate the residual tumor, which could not provide enough evidence for patients to undergo re-TURBT. The utMeMA could discriminate individuals

with residual tumors with a sensitivity of 93.3%, far better than urine cytology (27.3%) and FISH (64.3%). This enables clinicians to precisely choose target patients who are suitable for re-TURBT. As for monitoring recurrence, utMeMA showed a better sensitivity of 89.5%, compared with urine cytology (31.4%) and FISH (52.8%) (p value??). The novel method also performed better than Bladder EpiCheck™ in the monitoring of recurrence of low- (75% vs. 40%) and high-grade (93% vs. 89%) tumors [9]. High efficacy of monitoring recurrence may relieve patients from frequent cystoscopies.

In conclusion, the novel urine DNA methylation assay developed by Chen et al. showed satisfactory efficacy in detecting bladder cancer, especially in identifying early-stage, minimal, residual, and recurrent tumors. However, as the authors mentioned, the current results were published based on cross-sectional analysis. Therefore, the long-term efficacy needs to be verified by follow-up studies, especially the false-positive cases with later recurrence. It is widely known that combined detection via different types of markers may perform better than a single marker.

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