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UBC®Rapid Is Sensitive in Detecting High-Grade Bladder Urothelial Carcinoma and Carcinoma in situ in Asian Population

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Keywords
UBC®Rapid · Urine cytology · Biomarker · Bladder cancer · Cytokeratin 8 · Cytokeratin 18

Abstract
Objectives: Bladder cancer is a common type of malignancy. UBC®Rapid is a novel immunoassay detecting urine cytokeratin 8/18 protein. Studies have shown good accuracy of UBC®Rapid in detecting bladder cancer. UBC®Rapid has however to date not been evaluated in Asian patients. We evaluated UBC®Rapid in detecting Asian bladder cancer, together with urine cytology. ***Methods:*** In total, 112 patients were recruited from National University Hospital Singapore and 103 patients were included in this study, comprising 49 patients with bladder urothelial carcinoma (UC), 21 patients with bladder benign lesions, and 33 patients with normal bladder. All the bladder cancer and benign lesions were confirmed by histology. Voided urine was collected for UBC®Rapid test. The results were compared with urine cytology. ***Results:*** The bladder UC group had remarkably higher UBC®Rapid value than the control groups. The sensitivity, specificity, positive, and negative predictive value of UBC®Rapid in detecting bladder UC were 53%, 85.5%, 76.5%, and 66.8%, respectively. Those of urine cytology were 40.8%, 96.3%, 90.9%, and 64.2%, respectively. Adding UBC®Rapid to urine cytology increased sensitivity to 57.1% but decreased specificity to 81.8%. UBC®Rapid was sensitive in detecting high-grade bladder UC (61.1%) and carcinoma in situ (CIS) (72.7%), as compared to urine cytology for bladder UC (55.6%) and CIS (54.5%), respectively. ***Conclusion:*** UBC®Rapid is sensitive in detecting high-grade bladder UC and CIS in Asian population. It may be useful as an adjunct test to achieve better detection of bladder cancer.

Introduction

Bladder cancer is 6th human cancer in men and 17th cancer in women globally. In 2018, 550,000 new cases of bladder cancer were diagnosed worldwide [1]. Up to 85% of newly diagnosed bladder cancers are nonmuscle-invasive bladder cancer (NMIBC). NMIBC is the most recurrent type of human cancer (31–78% in 5 years) and long-term surveillance is mandatory after bladder-retaining therapies [2]. The diagnosis of bladder cancer and the postoperative surveillance of NMIBC involve cystoscopy and urine cytology. Cystoscopy is an invasive procedure which does not detect all bladder cancers. Urine cytology has high specificity but poor sensitivity and is particularly unreliable in detecting low-grade, low-stage tumors

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because the cytopathological features of malignancy are less prominent in those tumor cells [3]. Thus, continuous effort has been made to look for biomarkers to improve the diagnosis and surveillance of NMIBC.

UBC®Rapid is a point-of-care (POC) immunoassay measuring cytokeratin 8 and 18 protein (CK8/18)-soluble fragment in urine [4]. Two types of UBC®Rapid tests are available, a nonquantitative dichotomized test by visualization of a reaction band intensity and a quantitative test determining CK8/18 protein concentration [4]. CK8/18 protein are encoded by gene *KRT8* and gene *KRT18*, respectively. In bladder, CK8/18 are abundantly expressed in the cytoplasm of urothelial cells and function as intermediate filament-forming proteins. CK8/18 are key components of cytoskeleton structure [5]. Soluble fragments of CK8/18 are released into urine from urothelial tumors due to apoptosis and necrosis. Elevated levels of CK8/18-soluble fragments are an indication of underlying bladder cancer activity [4]. Increasing evidence suggests that UBC®Rapid is a promising test in detecting bladder cancer [6–9]. Thus far, all the clinical evaluations of UBC®Rapid studies completed are in Western population. Given the possible genetic variations of gene *KRT8* and gene *KRT18* in different ethnic groups, it is essential to expand the evaluation of UBC®Rapid to various human populations [10]. In addition, only a couple of papers have compared UBC®Rapid with urine cytology, the most clinically utilized urine-based diagnostic test of bladder cancer. Hence, we evaluated the quantitative UBC®Rapid test in detecting Asian bladder cancer, in parallel with urine cytology.

Methods

Study Population

In total, 112 patients with suspected bladder cancer or patients on bladder cancer surveillance were recruited from patients presented to Urology of National University Hospital Singapore during February 2018 to February 2020. Among them, 76 patients had bladder tumors or suspicious bladder findings on flexible cystoscopy and they underwent transurethral resection (TUR) or biopsy of bladder lesions. The other 36 patients had normal flexible cystoscopy and upper tract imaging. Exclusion criteria include current urinary tract infection, current gross hematuria, recent urinary tract instrumentation (catheterization, cystoscopy, intravesical therapy within 2 weeks), and concurrent urinary tract malignancy. Institutional Review Board approval was obtained for the study. Informed consent was taken from each patient.

In the 76 patients who underwent TUR or biopsy of bladder lesions, 74 patients had both urine cytology result and UBC®Rapid result for comparison. Among the 74 patients, there were 53 cases of histologically proven malignancy. These comprised 49 bladder UC, 1 concurrent prostate adenocarcinoma and bladder UC, 1 left distal ureter UC, 1 prostate adenocarcinoma, 1 bladder MALT lymphoma. Histology also revealed 21 cases of bladder benign lesions, including urothelial papilloma, inverted urothelial papilloma, nephrogenic adenoma, cystitis glandularis, granulomatous inflammation, etc. In the 36 patients with normal cystoscopy and urinary upper tract imaging, 33 had urine cytology and UBC®Rapid results. Thus, the matched UBC®Rapid results and voided urine cytology results from 103 patients were used for further analysis, comprising 49 patients with bladder UC, 21 patients with bladder benign lesions, and 33 patients with normal bladder. Histology grading and staging of the bladder UC are based on the 2004 WHO classification and the 2009 TNM classification, respectively.

Qualitative UBC®Rapid Test and Urine Cytology

Midstream urine (about 50 mL) was collected before flexible cystoscopy or TUR and subsequently aliquoted for UBC®Rapid assay and urine cytology. Urine specimens were stored at room temperature and used for UBC®Rapid test within a few hours. UBC®Rapid test was carried out based on the manufacturer's instructions (IDL Biotech, Bromma, Sweden). The quantitative analysis was performed by the photometric POC system Concile Omega 100 reader (Concile GmbH, Freiburg, Germany). Each urine specimen was measured twice. Additional tests were performed as needed and the results were averaged. UBC®Rapid test value >10 μg/L is considered as UBC®Rapid positive based on the manufacturer's recommendation. Urine cytology was reported by cytopathologists at National University Hospital Singapore. Reports of "high-grade urothelial carcinoma" or "highly atypical cells suspicious for high-grade urothelial carcinoma" are considered as urine cytology positive for UC.

Calculation and Statistical Analysis

The detection range of UBC®Rapid quantitative analysis is 5–300 μg/L. Any reading below 5 is shown as "<5" μg/L. Any reading above 300 μg/L is shown as ">300" μg/L. To estimate the mean and standard deviation (SD) of the UBC®Rapid values, we used 4 μg/L arbitrarily when the assay showed the reading value of "<5" μg/L and used 301 μg/L arbitrarily when the assay showed the reading value ">300" μg/L.

One-way analysis of variance (ANOVA) (Levene's test) was used to calculate whether the mean UBC results of various study groups were significantly different. Tukey HSD test was used to conduct pairwise comparisons and found that the mean result of each study group significantly differed from one another. $p < 0.05$ is considered as statistically significant.

Results

Table 1 summarizes the patient and tumor characteristics of the 3 study groups (bladder UC, benign lesion, and normal bladder group). As expected, the patients were predominately elderly, male, and Asian. All the patients had upper urinary tract imaging (computed tomography or ultrasound), flexible cystoscopy, voided urine cytology, and UBC®Rapid test. The UBC®Rapid

Table 1. Patient and tumor characteristics of bladder UC, bladder benign lesion, and normal bladder groups

Variable	UC (*n* = 49)	Benign (*n* = 21)	Normal (*n* = 33)
Male gender, *n* (%)	42/49 (85.7)	18/21 (85.7)	26/33 (78.8)
Mean age in years (SD)	69.3 (8.2)	72.2 (10.3)	66.5 (8.3)
Race, *n* (%)			
Chinese	37/49 (75.5)	16/21 (76.2)	26/33 (78.8)
Malay	7/49 (14.3)	2/21 (9.5)	3/33 (9.1)
Indian	4/49 (8.2)	1/21 (4.8)	0/33 (0)
Others	1/49 (2)	2/21 (9.5)	4/33 (12.1)
Tumor stage, *n* (%)			
CIS	2 (4.1)		
Concomitant CIS	9 (18.3)		
Ta	29 (59.2)		
T1	11 (22.4)		
T2	7 (14.3)		
Tumor grade, *n* (%)			
LG	13 (26.5)		
HG	36 (73.5)		

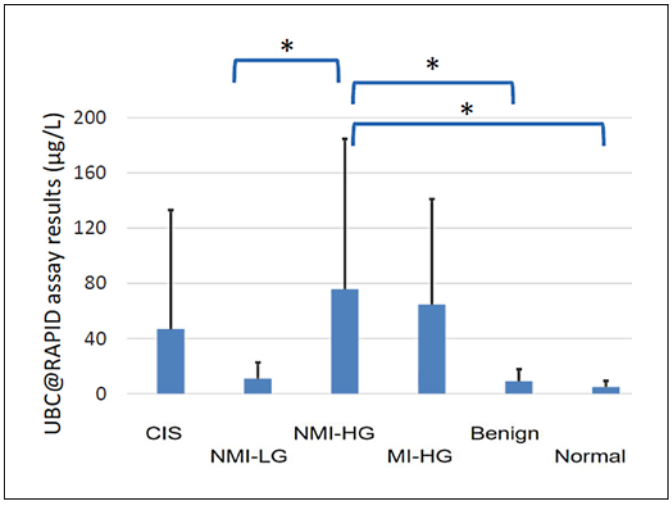

Fig. 1. UBC®Rapid values (mean and SD) of bladder CIS, NMI-LG, NMI-HG, MI-HG, benign bladder lesion, and normal bladder groups. Asterisk denotes that there is significant difference between the mean UBC®Rapid values of the 2 groups.

results of bladder UC, benign lesion, and normal bladder group were 50.8 ± 86.6 µg/L (mean ± SD), 8.9 ± 8.8 µg/L, and 4.8 ± 4.5 µg/L, respectively. Thus, the bladder UC group had remarkably higher UBC®Rapid result than bladder benign lesion group and normal bladder group (p = 0.0015, one-way ANOVA), suggesting that UBC®Rapid test is capable of detecting bladder UC. To investigate whether bladder UC with various histology grades and stages have different urine concentration of CK8/18, we looked into the UBC®Rapid results of bladder carcinoma in situ (CIS) (11 patients), nonmuscle invasive (NMI)-low grade (LG) (13 patients), NMI-high grade (HG) (20 patients), and muscle invasive (MI-HG) (5 patients). The mean values and SDs of UBC®Rapid test of these groups were 46.8 ± 86.4 µg/L (CIS), 10.8 ± 11.9 µg/L (NMI-LG), 75.6 ± 109 µg/L (NMI-HG), and 64.5 ± 76.6 µg/L (MI-HG), respectively, while those of bladder benign lesion and normal bladder group were 8.9 ± 8.8 µg/L and 4.8 ± 4.5 µg/L (Fig. 1). One-way ANOVA suggested that the mean UBC®Rapid results of the 6 groups were significantly different (p = 0.0004). Tukey HSD test showed that the NMI-HG group had a significantly higher mean than that of the NMI-LG, bladder benign lesion, and normal bladder groups (p = 0.027, p = 0.006, p = 0.005, respectively) while there was no significant difference among the other groups pairwise (Fig. 1).

Table 2 shows the performance of UBC®Rapid assay versus urine cytology in detecting bladder cancer. Overall, UBC®Rapid tests had lower specificity (85.5 vs. 96.3%) but higher sensitivity (53 vs. 40.8%) compared to urine cytology. Ninety percent of cytology-positive bladder UC were also UBC®Rapid positive, suggesting there was good concordance between the 2 diagnostic tools (Fig. 2). Ten cases of bladder UC were only detectable by either UBC®Rapid (8 patients) or cytology (2 patients). In the 8 cases of UBC®Rapid-positive only patients, 2 cases were high-grade UC with CIS, 2 cases were high-grade UC, and 4 cases were low-grade UC. In the 2 cases of cytology-positive only patients, both were high-grade UC. Twenty-one cases (42.8%) of UC were nondetectable by either type of tests, including 9 case of LG tumors, 9 cases of HG tumors, 2 cases of HG tumors with CIS, and 1 case of CIS.

Table 2. Performance of UBC®Rapid tests and urine cytology in detecting bladder cancer

	UBC®Rapid	Urine cytology	UBC®Rapid and urine cytology
Sensitivity, %	53	40.8	57.1
Specificity, %	85.5	96.3	81.8
Positive predictive value, %	76.5	90.9	77.8
Negative predictive value, %	66.8	64.2	63.2

Table 3. The sensitivity of UBC®Rapid and urine cytology in various stages and grades of bladder cancer

	UBC	Cyto	UBC and cyto
Tumor stage, *n* (%)			
CIS	72.7	54.5	72.7
Ta	34.8	17.4	39.1
T1	70	60	70
T2	60	80	80
Tumor grade, *n* (%)			
LG	30.8	0	30.8
HG	61.1	55.6	66.7

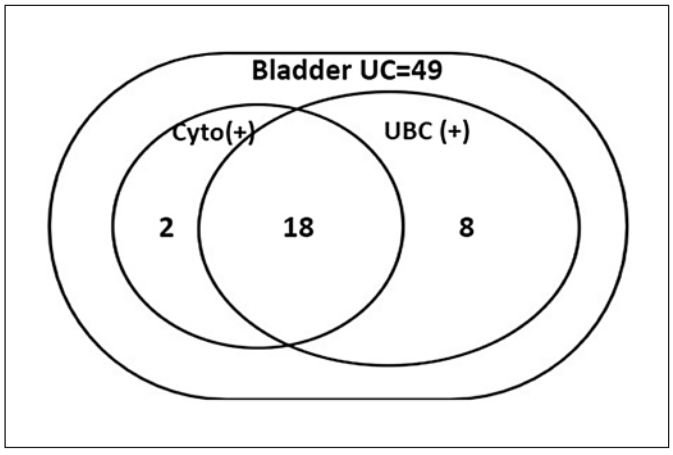

Fig. 2. Venn diagram of UBC®Rapid-positive bladder UC (26 cases) and cytology-positive bladder UC (20 cases). Note that 21 bladder UC were nondetectable by either UBC®Rapid or urine cytology.

We compared the sensitivity of UBC®Rapid and urine cytology in various grades and stages of bladder cancer (Table 3). UBC®Rapid was more sensitive (30.8%) than urine cytology (0%) in detecting low-grade bladder UC and equally sensitive (61.1%) in detecting high-grade bladder UC as urine cytology (55.6%). UBC®Rapid was also more sensitive in detecting CIS (72.7%), Ta tumors (34.8%) and T1 tumors (70%) compared to urine cytology (54.5% for CIS, 17.4% for Ta, 60% for T1) but less sensitive (60%) in detecting T2 bladder UC compared to urine cytology (80%). Lastly, adding UBC®Rapid to urine cytology increased sensitivity in detecting bladder UC from 40.8% to 57.1% but reduced the specificity from 96.3% to 81.8% (Table 2). The benefit of adding UBC®Rapid to urine cytology was more pronounced in CIS (from 54.5% to 72.7%) and low-grade tumors (from 0% to 30.8%) but less in high-grade tumors (from 55.6% to 66.7%), Ta, and T1 tumors (Table 3).

Discussion

Numerous molecular biomarkers for bladder cancer diagnosis and surveillance have been studied. Some assays have been approved by the Food and Drug Administration (FDA), such as BTA (bladder tumor antigen) stat®/BTA TRAK®, NMP22 (nuclear matrix protein)/BladderChek®, UroVysion™, and ImmunoCyt™/uCyt™ [11–14]. To date however, none of these markers have been accepted for diagnosis or surveillance of bladder cancer in clinical practice. Urine cytology remains the only urine-based diagnostic test in clinical practice currently, despite the significant progression on biomedical science and technology during the last few decades.

Similar to BTA and NMP22 tests, UBC®Rapid is a POC test. UBC®Rapid is straightforward to perform and fast to obtain result (less than15 min). There are some obvious advantages of UBC®Rapid compared to BTA and NMP22 tests. For example, BTA test results are completely unreliable if trace amount of blood (150 erythrocytes/μL) is present in the urine specimen [15]. NMP22 test is significantly less sensitive in detecting bladder cancer than UBC®Rapid (12.9–16.4% vs. 60.7–61.3%) in the head-to-head comparison [16, 17]. Considering the main issue of urine cytology is low sensitivity, UBC®Rapid may stand a better chance to complement urine cytology and improve bladder cancer diagnosis compared to other POC urine assays.

Our study showed good performance of qualitative UBC®Rapid in detecting bladder cancer in Asian population. The sensitivity (53%) and specificity (85.5%) in our study are comparable to those reported in Western population (sensitivity ranging from 46.2% to 78.4% and specificity ranging from 82.4% to 97.4%) [4, 7, 16–21]. Thus, UBC®Rapid may be useful in detecting bladder cancer in Asian population. Within our bladder UC group, NMI-HG tumors had significantly higher urine cytokeratin 8/18 protein levels than low-grade tumors (NMI-LG), likely reflecting that high-grade tumor may have greater degree of cancer tissue apoptosis and necrosis, thereby releasing more soluble cytokeratin 8/18 fragments into urine. In HG tumors however, there was no significant difference on urine cytokeratin 8/18 level between the MI and NMI tumors as muscular layer invasion in bladder may not necessarily increase soluble cytokeratin 8/18 concentration in urine [6].

Consistent with the literature, our study showed UBC®Rapid had overall higher sensitivity than urine cytology in detecting bladder UC (53 vs. 40.8%), especially in detecting CIS (72.7 vs. 54.5%), HG tumor (61.1 vs. 55.6%), and LG tumors (30.8 vs. 0%) [6, 16, 17]. Combining UBC®Rapid and urine cytology together increased the overall sensitivity compared to urine cytology alone in detecting bladder UC (57.1 vs. 40.8%) and in particular in detecting CIS (72.7 vs. 54.5%), HG tumor (66.7 vs. 55.6%), and LG tumors (30.8 vs. 0%). Eight (16.8%) cases of bladder UC were detectable by UBC®Rapid but not urine cytology, including 2 cases of HG tumor and 2 cases of HG tumor with CIS. Thus, UBC®Rapid could be used as an adjunct investigation to improve the detection of bladder cancer, to achieve earlier diagnosis and better treatment outcome. It is also clear that UBC®Rapid or urine cytology alone or combined is not sufficient to replace cystoscopy as 21 (42.8%) cases of UC were nondetectable by either type of tests.

Conclusion

Our study demonstrated good performance of UBC®Rapid in detecting bladder cancer in Asian population. UBC®Rapid is more sensitive and less time-consuming compared to urine cytology. While urine cytology will remain the main urine-based test in diagnosing bladder cancer clinically in the near future due to its high specificity, POC assays like UBC®Rapid may be useful as an adjunct test to achieve better bladder cancer detection. More clinical trials will help to investigate the value of UBC®Rapid in clinical application of bladder cancer.

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Statement of Ethics

This study was approved by the Institutional Review Board of National University Hospital Singapore (No. 2017/00486). Subjects have given their written informed consent to participate in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Kesavan Esuvaranathan and Zhijiang Zang conceptualized this study. Zhijiang Zang, Nisha Raishmi Kesavan, and Kesavan Esuvaranathan recuited the patients. Zhijiang Zang and Nisha Raishmi Kesavan performed the tests and data analysis. Zhijiang Zang drafted the manuscript. All authors approved the results and the final manuscript.

Data Availability Statement

The data that support the findings of this study are not publicly available as they contain information that could compromise the privacy of research participants. But the data are available from the corresponding author upon request.

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