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Clinical-Bladder cancer

Evaluation of URO17® to improve non-invasive detection of bladder cancer

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Abstract

Background: : The gold standard for detecting bladder cancer is cystoscopy with biopsy or transurethral resection confirming histologic diagnosis. URO17® employs a chromogenically labeled monoclonal antibody to keratin 17 (k17), an intermediate filament cytoskeleton molecule associated with bladder, pancreatic, and cervical cancers. Preliminary studies evaluating k17 demonstrated a high sensitivity and specificity for the detection of bladder cancer, supporting the need for further study.

Objective: To evaluate the sensitivity and specificity of URO17.

Methods: This is a cross-sectional study of participants undergoing urologic procedures between July 6, 2018 and July 17, 2019 at a single institution. Patients undergoing cystectomy, endoscopic bladder and/or upper tract procedure for probable urothelial carcinoma comprised cases; patients undergoing urologic procedures for other reasons comprised the control group (i.e. prostatectomy, nephrectomy, etc.). Voided urine samples were at the time of procedure; a minority of participants underwent multiple resections in the study period, thus, as many as three urine samples were taken from any given participant. Samples were distributed for blinded testing with URO17. Sensitivity and specificity were calculated.

Results: In 152 participants and 167 samples, URO17 demonstrated an overall sensitivity of 90% and 92% and a specificity of 88% and 87%, respectively. In 76 participants and 91 samples from patients with suspected urothelial carcinoma, the sensitivity was 90% and 92%, and the specificity was 50% and 54%, respectively. No controls demonstrated a positive URO17 result, and URO17 superseded urine cytology detection of low-grade and high-grade Ta. False positive results were associated with inflamed tissue or urothelial atypia on histology; the large majority had a history of intravesical therapy.

Conclusion: Limitations include cross-sectional design and convenience sampling. URO17 may improve sensitivity of urine cytology in the detection of urothelial cancer, though further study is required to refine the application of this biomarker in clinical practice.

Keywords: Urinary biomarkers; Bladder cancer; Cytology

1. Background

Nonmuscle-invasive bladder cancer (NMIBC) is challenging to monitor. Most patients with urothelial carcinoma present with NMIBC; high recurrence [1−3] and progression [4] rates require patients to undergo frequent cystoscopies with cytology.

Cystoscopy is the gold standard for bladder cancer diagnosis but is invasive and exposes the patient to procedural risk [5,6]. Urine cytology is noninvasive and demonstrates high specificity (99%), but with sensitivity as low as 34% [7] and high interobserver variability [8−10], its significance in the detection of bladder cancer may be limited [6,11,12]. Thus, our current methods of monitoring patients

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with NMIBC may result in diagnostic uncertainty leading to further biopsy or transurethral resection (TUR) and the risk of missing significant cancers.

A sensitive, noninvasive modality to detect bladder cancer may improve patient care and adherence, reducing bladder cancer morbidity and mortality. A number of non-invasive commercial tests have been developed demonstrating variable sensitivity and specificity [6,7,13], but none are sufficiently effective in clinical practice [14]. The URO17® test utilizes a promising technology to detect urothelial lesions via Keratin 17 (K17) staining in urine cytology specimens. Keratin 17 is an intermediate filament that has shown to be an important oncoprotein that binds and exports $p27^{KIP1}$ from the nucleus to cytoplasm, where it is degraded, thus bypassing G_1-S cell phase inhibition and resulting in sustained cell proliferation [Escobar-Hoyos et al., 2015, Cancer Research]. In a prior study, the URO17 test using K17 immunocytochemical detection of urothelial carcinoma in urine cytology samples demonstrated high sensitivity and specificity in recurrent urothelial carcinoma [15,16] and in urothelial carcinoma from patients with hematuria [16,17].

In this study, we aimed to evaluate the performance characteristics of URO17 to improve noninvasive detection of bladder cancer.

2. Materials and methods

2.1. Design and participants

This cross-sectional study was conducted on participants undergoing procedures for various urologic malignancies at a single institution between July 6, 2018 and July 17, 2019. The experimental group included patients undergoing cystectomy, endoscopic bladder and/or upper tract procedure for probable urothelial carcinoma comprised cases. Those undergoing urologic procedures for other reasons comprised the control group (i.e., prostatectomy, nephrectomy, etc.). Each participant provided informed consent.

2.2. URO17 immunocytochemistry

After collection, voided urine samples for URO17 test were distributed to the testing laboratory for blinded URO17 testing (processed and fixed to slides, stored between 2-8 degrees Celsius, and stained in weekly batches [17]). The median time from procedure to distribution was 3 days (IQR: 2, 6). Samples were centrifuged at 1000 g for 10 minutes, the pelleted cells were resuspended in 20 mL of PreservCyt Solution (Hologic Marlborough, MA), and the cells were transferred onto charged glass slides using a ThinPrep 2000 processor (Hologic, Marlborough, MA). The slides were stained using a Dako auto-stainer Link48 (Agilent technologies, Carpinteria, CA). Endogenous peroxidase activity was blocked using EnVision Flex wash peroxidase-Blocking reagent (Agilent technologies, Carpinteria, CA). Following incubation with URO17 anti-Keratin 17 antibody (KDx Diagnostics Inc., San Jose, CA; 0.3 ug/mL final concentration), slides were processed by a direct polymer-based immunoperoxidase method using EnVision Flex HRP (Agilent technologies, Carpinteria, CA), developed in EnVision Flex DAB+ chromogen (Agilent technologies, Carpinteria, CA), and counterstained with hematoxylin. Slides were dehydrated in graded ethanols and cover-slipped. Cytopathologists evaluated the blinded slides and the samples with 5 or more K17-positive urothelial cells with cytoplasmic and/or nuclear staining intensity of 2+ and above were determined as positive for URO17.

2.3. Outcome measurements and statistical analysis

Clinical and demographic information was collected to characterize patient history before URO17 procedure, including initial bladder cancer diagnosis and treatment with descriptive statistics using tabulation, Median IQR, and percentages. Available cytology results within 8 weeks of URO17 testing and with no treatment in between the 2 tests was recorded. We evaluated the sensitivity and specificity of URO17 in the tradiational way based on a histopathologic diagnosis of the presence or absence of bladder cancer in multiple modalities: 1) in all samples regardless of operation type, 2) in all suspected UC samples, and 3) in initial URO17 evaluations only.

3. Results

A total of 152 participants underwent URO17 testing (76 cases, 76 controls). The median age of cases was 70 (IQR: 64, 76), and the median time from initial bladder cancer diagnosis was 9 months (IQR: 3, 33). The median time from procedure to distribution was 2 days (IQR: 1, 5). The large majority underwent endoscopic bladder procedures (90%). Over half (54%) of patients received intravesical therapy before the study; the median time between intravesical therapy and study participation was 3 months (range: 0−129 months). Over one-third of patients experienced urothelial carcinoma recurrence. The median age of controls was 65 (IQR: 61, 70), and 86% underwent prostatectomy (86%). Clinical information for cases and controls are summarized in Tables 1 and 2, respectively.

In 167 samples from both cases and controls, URO17 demonstrated an overall sensitivity of 90% and a specificity of 88%. In 91 samples from patients with suspected urothelial carcinoma, the sensitivity was 90% and the specificity was 53% (Figure 1). In the 76 participants, 91 samples, with suspected bladder cancer, the sensitivity was 90% and the specificity was 59%. In the 76 initial samples, the sensitivity was 89% and the specificity was 52%. In this study, PPV was consistent between all populations, but NPV ranged from 67% to 100% based on the population. No controls demonstrated a positive URO17 result. URO17

Table 1
URO17 collection at procedure for suspected or known urothelial carcinoma

Characteristic, Median (IQR) or n (%)	
No. of participants	n = 76
Sex	
Male	56 (74%)
Female	20 (26%)
Race	
White	58 (76%)
African American/Black	3 (3%)
Asian/Pacific Islander	6 (8%)
Other/Mixed/Unknown	7 (9%)
Declined	2 (2%)
Ethnicity	
Hispanic	5 (7%)
Not Hispanic	67 (88%)
Declined	4 (5%)
Smoking status	
Never	24 (32%)
Former	49 (66%)
Current	1 (1%)
If former or current, pack-years, median (IQR)	12 (6, 30)
Procedure at time of URO17 collection	
Radical cystectomy	4 (5%)
Partial cystectomy	2 (3%)
Partial urethrectomy	1 (1%)
Nephroureterectomy	1 (1%)
Endoscopic	68 (90%)
Histology	
Urothelial carcinoma	45
Variant	6
Benign urothelium	22
Clinical or pathologic stage, T	
T0	22
Tis	12
Ta	25
T1	11
T2	2
T3	1
T4	2
Pathologic stage, N	
NX	2
N0	4
N1-3	1
Grade	
HG	47
LG	8
PUNLMP/UPUMP	2
Initial diagnosis	
Bladder	69
Upper tract	6
Histology	
Urothelial carcinoma	73
Variant	2
Grade at initial diagnosis	
High-grade	54
Low-grade	19
PUMLMP	1
Stage at initial diagnosis	
Tis	12
Ta	39
T1	19
T2	7
T3	2
T4	1
History of intravesical therapy	41 (54%)
Months from most recent intravesical therapy, median (range)	3 (0, 129)
Received BCG	33
Received other types of intravesical therapy	
Mitomycin	6
Gemcitabine	9
Received more than one type of intravesical therapy	2
History of perioperative intravesical therapy	12
Months from most recent perioperative intravesical therapy, median (range)	11 (0, 35)
Atezolizumab (clinical trial)	2
History of recurrence	28 (37%)
Number of recurrences, median (range)	1 (1, 7)
Most recent recurrence	
Months from most recent recurrence, median (range)	7 (1, 44)
Bladder	25
Upper tract	3
Grade at most recent recurrence	
High-grade	23
Low-grade	3
UPUMP	1
Stage at most recent recurrence	
Tis	9
Ta	13
T1	5
T2	3
T3	1
T4	0
Cytology	
1 Nondiagnostic/unsatisfactory	0
2 Negative for high-grade urothelial carcinoma (NHGUC)	31
3 Atypical urothelial cells (AUC)	25
4 Suspicious for high-grade urothelial carcinoma (SHGUC)	1
5 High-grade urothelial carcinoma (HGUC)	16
6 Low-grade urothelial neoplasm (LGUN)	0
7 Other: primary and secondary malignancies and miscellaneous lesions	2
History of:	
Pelvic radiation	8
Neoadjuvant therapy	14
UTI	13
Recent UTI (within one month of procedure)	2

superseded urine cytology in detecting both low-grade and high-grade Ta. URO17 detected all cases classified as high-grade on cytology and discovered an additional 13 cases missed by cytology (defined as Paris 2 cytology, but had high grade disease at biopsy). Detection of lesions by URO17 organized by Paris System for Reporting Urinary Cytology [18] is presented in Table 3.

This study yielded 12 false positive and 6 false negative results. Eleven false positives occurred during initial URO17 testing, while one occurred on repeat testing. Eleven of 12 false positive cases demonstrated histologic findings consistent with granulomatous inflammation, foreign body giant cell reaction, necrosis, focal scarring, focal atypia, and/or squamous metaplasia. Ten of 12 participants

Table 2
Clinical information for controls

Characteristic, Median (IQR), or n (%)	
No. of participants	n = 76
Sex	
Male	72
Female	4
Race	
White	68
African American/Black	0
Asian/Pacific Islander	3
Other/Mixed/Unknown	5
Ethnicity	
Hispanic or Latinx	4
Days between kdx procedure and distribution	3 (2, 6)
Procedure of kdx collection	
Procedure of kdx collection	
Radical prostatectomy	65 (86%)
Histology	
Adenocarcinoma	60
PIN	1
Pathologic stage, pT	
pT0	0
pT1	0
pT2	26
pT3	36
pT4	1
TX	2
Pathologic stage, N	
NX	20
N0	37
N1	8
Gleason	
6	2
7	42
8	2
9	12
10	1
Nephrectomy	9 (12%)
Radical	5
Partial	4
Histology	
Clear cell RCC	3
Other	6
Pathologic stage, pT	
pTx	1
pT0	0
pT1	5
pT2	0
pT3	2
pT4	0
Pathologic stage, N	
NX	7
N0	1
N1	0
Endoscopic	2
Smoking status	
Never	47
Former	26
Current	3
If former or current, pack-years	9 (2, 17)
History of:	
Pelvic radiation	8 (10%)
Other cancer	4 (5%)
Bladder cancer	1 (1%)
Hematuria	7 (9%)

with false positive results received intravesical therapy at a median interval of 2 months (range: 0, 45) from time of URO17. If excluding those who received BCG in the 3 months prior to URO17, sensitivity of initial URO17 remained stable at 87.7% and specificity increased to 52.9%; these performance statistics change only slightly when those receiving any form of intravesical therapy within 3 months of URO17 were excluded (88.3% and 53.3%). Discordant URO17 results are summarized in Table 4.

4. Discussion

Overall, URO17 results correlated with pathology and demonstrated high sensitivity and specificity in the study population. URO17 was negative in all participants without bladder cancer. URO17 also detected variant lesions such as adenocarcinoma and neuroendocrine bladder tumors.

In patients with suspected bladder cancer, URO17 accurately detected 90% of cases. This is higher than the sensitivity exhibited by the majority of commercially available diagnostic tools used to detect bladder cancer in patients with established or suspected bladder cancer history (10%–85% by Hemastix, 47%–93% by NMP22, 53%–78% by BTA Stat, and 63%–85% by ImmunoCyt) [6]. The sensitivity of URO17 in our study was similar to Urovysion FISH, which detects 87% of bladder cancer (94% of muscle-invasive cancer) [19]. URO17 performed comparably with published literature on newer commercial testts such as Cxbladder [20], which demonstrate high sensitivity (>90%) [21,22,23].

URO17 detected many low- and high-grade Ta lesions missed on urine cytology. In this study, cytology was negative or atypical in 32 of 76 bladder cancer cases (43%). Cytology has been demonstrated in prior studies to miss over half of positive urothelial carcinoma cases [12] and to vary widely based on observers [9] and between institutions [8]. Therefore, the development and integration of ancillary tests, like URO17, has become an increasingly important and may help to stratify indeterminate diagnosis of atypical/suspicious category to more definite category of HGUC. Additionally, URO17 outperforms most tests in detecting low-grade lesions (88% detection of LG urothelial carcinoma and 50% detection of PUMLMP/UPUMP by URO17 vs. 38% of low-grade lesions by Hemastix, 25% by NMP22, 36% by BTA Stat, and 47% by ImmunoCyt) [6],

We observed 12 false positive results among cases; 11 of these were in initial tests and one was on repeat URO17 testing. Specificity of URO17 in this study for patients with history of bladder cancer (54%) was lower compared to other diagnostic tests in patients with bladder cancer (40%–90%) [6,24], with false positive cases associated with benign epithelial disturbances. However, the specificity of URO17 in all patients was significantly higher (88.5%) when compared to the history of bladder cancer population. These observations suggest that the relatively

(A)

	Disease +	Disease -	
URO17 +	56	12	68
URO17 -	6	93	99
	62	105	167
Sensitivity	0.903226		
Specificity	0.885714		

(B)

	Disease +	Disease -	
URO17 +	47	11	58
URO17 -	6	88	94
	53	99	152
Sensitivity	0.886792		
Specificity	0.888889		

(C)

	Disease +	Disease -	
URO17 +	56	12	68
URO17 -	6	17	23
	62	29	91
Sensitivity	0.903226		
Specificity	0.586207		

(D)

	Disease +	Disease -	
URO17 +	47	11	58
URO17 -	6	12	18
	53	23	76
Sensitivity	0.886792		
Specificity	0.521739		

(E)

	Disease +	Disease -	
URO17 +	20	3	23
URO17 -	0	5	5
	20	8	
Sensitivity	1		
Specificity	0.625		

Figure 1. Sensitivity and specificity of calculated using (A) all samples (B) initial kdx in all participants, (C) all samples in participants with urothelial carcinoma, (D) initial kdx in participants with urothelial carcinoma, (E) all repeated samples in participants with urothelial carcinoma. (Disease + includes variants, PUNLMP, and UPUMP lesions).

Table 3
Diagnosis of UC among patients stratified by Paris cytology classification and URO17 result (cases, initial kdx only)

	Total	LG Ta UC	HGTa UC	HG ≥T1 UC	Cis	PUNLMP/ UPUMP	Variant*	Negative biopsy†
2 Negative for high-grade urothelial carcinoma (NHGUC)								
Positive URO17	24	6	8	4	0	0	1	5
Negative URO17	7	1	0	0	0	0	0	6
3 Atypical urothelial cells (AUC)								
Positive URO17	16	1	5‡	4‡	2	0	0	4
Negative URO17	9	0	1	2§	1	1	0	4
4 Suspicious for high-grade urothelial carcinoma (SHGUC)								
Positive URO17	1	0	1	0	0	0	0	0
Negative URO17	0	0	0	0	0	0	0	0
5 High-grade urothelial carcinoma (HGUC)								
Positive URO17	16	0	2	4	7	1‖	0	2
Negative URO17	1	0	0	0	0	0	0	1
7 Other: primary and secondary malignancies and miscellaneous lesions								
Positive URO17	1	0	0	0	0	0	1	0
Negative URO17	1	0	0	0	0	0	0	1
Total	76	8	17	14	10	2	2	23
Positive URO17	58	7	16	12	9	1	2	11
Negative URO17	18	1	1	2	1	1	0	12
Sensitivity	90%	88%	94%	86%	90%	50%	100%	

* Variant histologies include UC with squamous differentiation, adenocarcinoma, neuroendocrine.
† Benign findings include squamous lined cyst (class 3), benign nephrogenic adenoma/metaplasia (class 7).
‡ Includes at least one patient with concomitant CIS.
§ Includes at least one patient with upper tract pathology.
‖ UPUMP.

Table 4
Clinical history after discordant URO17

Characteristic, Median (IQR) or n (%)	False positive	False negative	Overall
No. of participants	n = 12	n = 6	n = 18
Cases	12	6	18
Controls	0	0	0
Months from discordant URO17 to most recent chart review, median (range)	20 (9, 22)	21 (17, 22)	21 (9, 22)
Procedure at discordant URO17 collection			
Cystectomy or urethrectomy	0	1	1
Nephroureterectomy/ureterectomy	0	1	1
Endoscopic bladder or upper tract procedure	12	4	16
Alive	12	6	18
Dead	0	0	0
No evidence of disease	11	5	15
Recurrence after kdx	3	1	3
Months to recurrence, median (range)	10 (4, 15)	6 (6, 6)	6 (4, 15)
Number of recurrences, median (range)	1 (1, 1)	1 (1, 1)	1 (1, 1)
PUMLMP on TURBT	0	1	1
CIS on TURBT	2	0	2
History of intravesical therapy	10	2	12
Months from intravesical therapy, median (range)	2 (0, 45)	4 (0,8)	2 (0, 45)
BCG	7	1	8
Mitomycin	1	2	3
Gemcitabine	2	2	4
Multiple regimens	0	2	2
History of perioperative intravesical therapy	3	2	5
Months from perioperative intravesical therapy, median (range)	26 (3, 34)	2 (0, 4)	4 (0, 34)
History of:			
Pelvic radiation	2	0	2
Neoadjuvant therapy	1	1	2
UTI	4	2	6
UTI within one month of URO17	0	0	0

lower specificity due to false positive results in our study could be due to (1) Keratin 17 activation in regenerating urothelial cells due to structural tissue damage by a tumor, inflammation, or BCG therapy, (2) field effect and multifocal nature of urothelial carcinoma, or (3) presence of residual cancer including upper tract cancers. As such, the implications of positive URO17 results should be carefully considered with other clinical factors in patients with previously established reasons for inflammation or other conditions that may have caused trauma to the urothelium, warranting further study into the timing of intravesical therapy and performance of URO17. On the other hand, the negative URO17 still could provide assurance that an active cancer is unlikely be present which could help guide the physicians in the treatment decisions. Furthermore, relatively high PPV and NPV in all participants in the study suggest that the status of URO17 could be a useful in ruling out patients who are unlikely to have active urothelial cancer.

Though not reaching the 100% sensitivity reported by previous studies [15−17] we did find a high sensitivity. Compared to the initial studies on Keratin 17 demonstrating high sensitivity and specificity in recurrent cancers [15,16] this study may reflect more real-life conditions. In addition, our study population and design differed from recent UK study by Vasdev et al. [17] as we included a comparative group and used patients with hematuria as a baseline cohort and that our study examined bladder cancer patients who are being monitored for cancer recurrence where the Vasdev et al., study examined newly diagnosed bladder cancer patients from the hematuria population. The major difference between the 2 populations is that none of the newly diagnosed patients was subjected to prior therapy, including the BCG, which may have eliminated a major confounding factor for the false positive results.

URO17 is a relatively simple immunocytochemical test with relatively short run time (2−3 hours). Furthermore, the test utilizes the same urine cytology slides and autostainer that are used commonly in most pathology laboratories. Since the urine sample processing methods, such as Thinprep (Hologic), and the immunostaining methods in autostainers are well established efficient methods that have been utilized over many years with well-accepted workflow. Thus, in most cases, URO17 test does not require special training to perform in most pathology laboratories. The test still requires an evaluation by a trained cytopathologist, but this is again a method that has been well established for urine cytology. In fact, the ability for the same cytopathologist to read both the traditional urine cytology slides with

URO17 slides provides new opportunity to increase the accuracy of cytological examination of urine samples by providing useful clinical information on categorizing atypical and suspecious urine cytology samples.

Limitations include cross-sectional design and convenience sampling; thus, prospective studies will need to be conducted. While cytology uses a 7-stage system, URO17 may be limited by its dichotomous reporting. Future studies may involve correlating strength of staining and the number of K17 positive cells with clinical findings for a more nuanced assessment and improved specificity of urothelial neoplasms among patients with suspected urothelial carcinoma. Other specific potential areas of study include investigating URO17 as a diagnostic adjunct to cytology and the application of URO17 to detect variant bladder cancer histologies.

5. Conclusion

URO17 may improve sensitivity of urine cytology in the detection of urothelial cancer, though further study is required to refine the application of this biomarker in clinical practice.The combined interpretation of cytomorphology (high specificity) and URO17 (improves sensitivity) will yield a comprehensive result for patient management. Future studies also aim to examine the timing of intravesical therapy and false positive rates, which would be important to determine prior to the use of URO17 with cytology. Overall this is a promising urinary biomarker that would benefit from further study to optimize clinical integration.

Declaration of competing interest

SJ is co-founder, President, and COO of KDx diagnostics; NK is co-founder and CEO of KDx diagnostics.

CRediT authorship contribution statement

Sima P. Porten: Data curation, Investigation, Resources, Writing – original draft, Writing – review & editing. **Elizabeth Y. Wang:** Data curation, Formal analysis, Project administration, Writing – original draft. **Poonam Vohra:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **Peter R. Carroll:** Resources, Supervision, Writing – original draft, Writing – review & editing. **Sholeh Jahanfard:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Nam W. Kim:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing.

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