

The Diagnostic Performance of Cxbladder Resolve, Alone and in Combination with Other Cxbladder Tests, in the Identification and Priority Evaluation of Patients at Risk for Urothelial Carcinoma

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Study Need and Importance: The earliest sign of urothelial carcinoma (UC) is usually hematuria, yet only a small proportion of people with hematuria actually have UC. Therefore, many individuals needlessly undergo diagnostic work-up including cystoscopy, and incur unnecessary inconvenience, risk, cost and anxiety. To reduce this burden, the latest American Urological Association/Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction guidelines now state that cystoscopy may be deferred, with shared patient decision making, in low-risk patients. However, only 4.6% of patients meet the guideline definition for low risk, highlighting the need for a noninvasive test that segregates safely hematuria patients without UC from those at elevated risk. The Cxbladder tests were designed to meet this need by accurately ruling out patients who do not have UC. They are used in sequence (Triage, Detect then Resolve), with each positive test reflexing to the next to maximize the diagnostic yield. The final step (Cxbladder Resolve; CxbR) identifies patients with a high probability of high impact tumors (HIT, ie high-grade Ta, T1-T3 or Tis tumors).

What We Found: This study validated the performance of CxbR to identify patients with HIT, and the sequence of Cxbladder tests to risk-stratify patients. CxbR had high sensitivity (92.4%) and specificity (93.8%) for HIT, and the Cxbladder test sequence correctly ruled out 87.6% of patients who needed no further workup for UC (negative predictive value 99.4%). The diagnostic yield of sequential Cxbladder tests for any UC was 4.8-fold higher than guideline-based risk stratification. All patients with HIT were correctly assigned to prioritized evaluation.

Limitations: Limitations include the small proportion of patients with tumors and high proportion with microhematuria in the validation data set.

Interpretation for Patient Care: Cxbladder tests are already used routinely in some countries and have proven clinical utility to target resources more efficiently and spare patients unnecessary invasive testing, by safely risk-stratifying patients using a single urine sample. Cxbladder tests offer an accurate adjunct to the American Urological Association 2020 guidelines to improve risk stratification during assessment of hematuria and/or diagnosis of UC.

The Diagnostic Performance of Cxbladder Resolve, Alone and in Combination with Other Cxbladder Tests, in the Identification and Priority Evaluation of Patients at Risk for Urothelial Carcinoma

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Purpose: Cxbladder (Cxb) tests combine genomic biomarkers in urine with phenotypic and clinical data to classify hematuria patients into those at low/high probability of urothelial carcinoma (UC). Cxbladder Resolve (CxbR) is designed for use after Cxb Triage (CxbT) and Detect (CxbD), where CxbT-positive tests reflex to CxbD and CxbD-positive to CxbR to identify patients at high probability of high-impact tumors (HIT; high grade Ta, Tis or T1–T3). This study validated the diagnostic performance of CxbR in identifying HIT, and validated the algorithm of Cxb tests to segregate high-impact from low-impact tumors.

Materials and Methods: CxbR was developed in 863 hematuria patients in 3 studies in United States, Australia and New Zealand. CxbR, separately and combined with other Cxb tests, was validated in a prospective, observational U.S. study in 548 hematuria patients. All UC diagnoses were confirmed by histopathology.

Results: In the development data set, CxbR sensitivity was 92.4% (95% CI 83.3–96.7) and specificity 93.8% (95% CI 86.8–97.2) for identifying HIT within the high priority category. During external validation, sequential Cxb tests correctly ruled out 87.6% of patients from further workup (negative predictive value 99.4%); 100% of HIT were correctly identified (specificity 96.3%), and 3 low-grade tumors were missed. In both studies, all patients with HIT were correctly assigned to prioritized evaluation.

Abbreviations and Acronyms

AUA = American Urological Association

Cxb = Cxbladder

CxbD = Cxbladder Detect

CxbR = Cxbladder Resolve

CxbT = Cxbladder Triage

DDS = development data set

HIT = high impact tumors

KP = Kaiser Permanente

LIT = low impact tumors

NPV = negative predictive value

PDP = physician-directed protocol

UC = urothelial carcinoma

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Conflicts of interest/financial disclosures: Dr. Jay Raman has been a study investigator for trials sponsored by MDxHealth and Urogen Pharma LLC, and has provided advisory services to Urogen Pharma LLC. Dr. Yair Lotan has been a study investigator for Vessi Medical, CAPs Medical, Abbott, Cepheid, Pacific Edge, FKD, MDxHealth, Biocancell, GenomeDx Biosciences Inc., Storz, Photocure, Radera, Synergo, BMS, Urogen and Zymo, and has provided advisory services to C2I genomics, Photocure, AstraZeneca, Merck, Fergene, Abbvie, Cleveland Diagnostics, Nucleix, Amby, Seattle Genetics, Ferring Research, Hitachi, and Verity Pharmaceuticals. Dr. Badrinath Konety is a consultant to Ferring, Boston Scientific, Convergent Genomics and a clinical investigator for Merck, BMS, Pacific Edge and Photocure. Dr. Siamak Daneshmand has been study investigator for trials sponsored by Photocure, Janssen, Ferring, Taris, Pacific Edge, Johnson & Johnson, BMS, and QED and has provided advisory services to Janssen, Ferring, Photocure, Taris, Spectrum, Pacific Edge, QED, Abbvie, Johnson & Johnson, Seattle Genetics, Nucleix, Aduro, and BMS. Dr. Sima Porten is on the advisory board of ProTara, Kdx, has consulted for Fergene and Photocure and has provided research support to Genome Dx Biosciences.

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Conclusions: CxbR has high sensitivity and specificity, correctly identifying all HIT. Sequential Cxb tests accurately segregate patients with a low vs high probability of HIT, focusing resources on those patients, with a diagnostic yield 4.8-fold higher than American Urological Association guideline stratification.

Key Words: biomarkers, hematuria, predictive value of tests, sensitivity and specificity, urinary bladder neoplasms

HEMATURIA is a common clinical presentation and key symptom of urothelial carcinoma (UC).^{1,2} American Urological Association (AUA) guidelines recommend clinical workup for patients with hematuria,³ but only 0.5%–5% of patients with asymptomatic microhematuria and 10%–15% with gross hematuria have confirmed UC.^{4,5} Most patients referred for evaluation are intermediate or high-risk for whom cystoscopy is recommended,³ but some of the investigated patients will return an inconclusive result, such as atypical cytology or equivocal cystoscopy.⁶ Conversely, many patients with microhematuria are not referred for evaluation even if they are at high risk.^{4,7}

There is, therefore, an unmet need for an accurate noninvasive urinary biomarker test that can rule out patients without cancer and identify patients at greatest risk of UC. Such a test would have high clinical utility by reducing the diagnostic burden on individuals without UC and prioritizing evaluation for those at risk of more advanced UC.

The family of Cxbladder (Cxb) tests (Pacific Edge, Dunedin, New Zealand) uses multiplexed mRNA biomarkers to guide the management of UC from diagnosis through to surveillance for disease recurrence (table 1).^{8–12} Specifically, Cxbladder Triage (CxbT) is a rule-out test with high sensitivity and high negative predictive value (NPV) to identify patients with a low likelihood of UC who may avoid unnecessary invasive investigations. Cxbladder Detect (CxbD) has high sensitivity and specificity for the identification of patients who require further workup for UC.¹² Cxbladder Resolve (CxbR) was developed for use after both CxbT and CxbD to accurately segregate test-positive patients likely to have high impact tumors (HIT). Further details on Cxb tests are provided in the supplementary material (<https://www.jurology.com>).

The current study aimed to validate, firstly, the diagnostic performance of CxbR to identify HIT and, secondly, CxbR in combination with CxbT and CxbD using a single urine sample at the time of initial investigation as part of a proposed new algorithm, where CxbT-positive tests reflex to CxbD and CxbD-positive to CxbR (fig. 1).

PATIENTS AND METHODS

Design

CxbR was developed and internally validated in a cohort of patients (863) with hematuria undergoing urological investigation for UC in 3 separate prospective studies in

the United States, Australia and New Zealand (supplementary methods and supplementary table, <https://www.jurology.com>). External validation of CxbR and validation of the algorithm (CxbT reflexing to CxbD reflexing to CxbR) was undertaken on an independent cohort from a prospective, observational study (548) at Kaiser Permanente (KP) in Southern California (supplementary methods, <https://www.jurology.com>).

The study protocols were approved by institutional review boards at participating centers (KP Independent Review Board No. 10477). All studies were performed in accordance with the principles of the Declaration of Helsinki, International Conference on Harmonization and Good Clinical Practice. Informed consent was obtained from all patients prior to enrollment.

Patients

For the development data set (DDS), all consecutive hematuria patients were screened for eligibility. Patients were aged ≥ 18 years in 2 studies, ≥ 45 years in another, and were scheduled to undergo cystoscopy for possible UC because of gross or microscopic hematuria (see supplementary table, <https://www.jurology.com> for full selection criteria). All patients underwent the standard workup.

The external validation cohort included hematuria patients undergoing routine workup at a KP center (supplementary methods, <https://www.jurology.com>). Patients were excluded for a prior history of UC, symptoms indicative of a current urinary tract infection, a confirmed diagnosis of prostate carcinoma or pregnancy (see supplementary table, <https://www.jurology.com>). Clinicodemographic characteristics were collected as determined by the routine clinical workup. Patients with incomplete data for Cxb tests or histopathological diagnosis were excluded from this analysis.

Cxb Testing

A single midstream urine sample was collected from each consenting patient and analyzed as a commercial Cxb test.^{10,12} Each Cxb test uses quantitative reverse transcription polymerase chain reaction to measure the expression of a common backbone of 5 genotypic biomarkers (MDK, CDK1, IGFBP5, HOXA13 and CXCR2) and combines these with specific phenotypic biomarkers and clinical characteristics to derive a test score.⁸ Thresholds based on these scores have been previously defined and validated for CxbT and CxbD (table 1 and supplementary methods, <https://www.jurology.com>).^{9,10,12} Samples from the patients testing positive on CxbT and CxbD underwent the CxbR test, which integrates the expression of the genotypic biomarkers with age and smoking history in a novel algorithm to identify patients with a high likelihood of HIT requiring priority workup, and segregate other patients into those who can be

Table 1. Summary of published performance of all Cxb tests and clinical applications

Function	Evaluation of Hematuria		Monitoring for Recurrence Cxb Monitor ³
	CxbT ¹⁰	CxbD ¹²	
Genomic markers	To rule out low-risk pts MDK, CDC2, IGFBP5, HOXA13 and CXCR2	To detect pos pts MDK, CDC2, IGFBP5, HOXA13 and CXCR2	To rule out low-risk pts MDK, CDC2, IGFBP5, HOXA13 and CXCR2
Phenotypic or clinical characteristics	Age, gender, smoking and hematuria status	Age and smoking history	Previous tumor status (primary or recurrent) and time since previous tumor
Detection categories*	Low probability vs not low probability of bladder Ca	Low vs elevated vs high risk of bladder Ca	High priority vs PDP vs observation vs Low probability vs not low probability of bladder Ca
Sensitivity	95%	82%	92.4%
NPV	99%	97%	93.8%
Specificity	Not applicable	85%	99.7%
			Not applicable

* Based on algorithmically defined scores and thresholds. Unqualified sensitivity, specificity and NPV are not defined for a test with 3 outcomes. Performance figures are bootstrapped values from DDS, where true positives are HIT and test positives are assigned to high-priority evaluation category, with sensitivity, specificity and NPV as stated.

managed by observation and those can be worked up according to their physician’s specification (ie physician-directed protocol [PDP]). Ongoing followup in these patients was based on clinical indication/need (eg persistence of hematuria, suspicion of other urological diagnoses).

All patients underwent a standard workup to determine clinical outcome for UC based on histopathology-validated cystoscopic examination. Disease stage was classified using tumor-node-metastasis criteria.¹³ Grade was classified according to local pathology practice, World Health Organization (WHO) grading criteria (1973)¹⁴ or WHO/International Society of Urological Pathology consensus classification (1998).¹⁵ HIT included high-grade Ta, T1-T3 and Tis. Low impact tumors (LIT) included low-grade noninvasive papillary carcinoma (Ta) and papillary urothelial neoplasm of low malignant potential. Performers/readers of the Cxb tests were blinded to cystoscopic/histopathological results and physicians had no access to Cxb results.

Sequential use of Cxb tests was investigated using the algorithm in figure 1. CxbT and CxbD maximize safe rule-out of patients at low probability of disease (based on high sensitivity and NPV), and CxbR segregates patients at high probability of HIT (based on high sensitivity and specificity).

Statistical Analysis

Development and internal validation of CxbR were undertaken on the DDS (patients in 3 studies from the United States, Australia and New Zealand). As CxbR has 3 possible outcomes (high priority, PDP, or observation), 2 parameters of sensitivity and specificity were assessed: 1) sensitivity/specificity (HIT), based on the proportion of HIT in the samples assigned to the “high priority” category; 2) sensitivity/specificity (UC), based on the proportion of any UCs assigned to “high priority” or “PDP”. Other performance parameters were calculated using standard methods.^{16,17} Data analysis used R-3.5.1 software.¹⁸ Bootstrap methodology was used to correct for bias in the observed values and calculate confidence

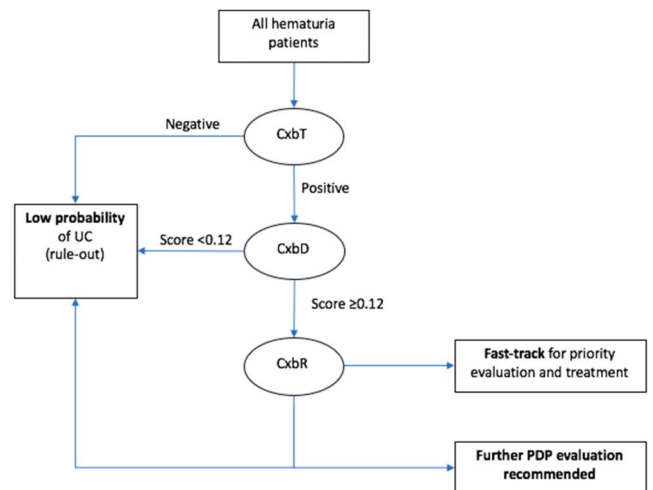


Figure 1. Proposed clinical algorithm for concatenated use of Cxb tests in assessment of patients with hematuria.

Table 2. Demographic characteristics of patients in DDS for CxbR, and those in external validation data set for CxbR who also underwent testing with concatenation of CxbT, CxbD and CxbR

	Development Data CxbR (863 pts)		Independent Data Tested with CxbT, CxbD and CxbR (548 pts)	
	Males (634 pts)	Females (229 pts)	Males (276 pts)	Females (272 pts)
No. yrs age (%):				
0–30	6 (0.9)	5 (2.2)	3 (1.1)	5 (1.8)
>30–40	9 (1.4)	7 (3.1)	14 (5.1)	13 (4.8)
>40–50	50 (7.9)	39 (17.0)	35 (12.7)	36 (13.2)
>50–60	110 (17.4)	64 (27.9)	48 (17.4)	76 (27.9)
>60–70	170 (26.8)	57 (24.9)	79 (28.6)	77 (28.3)
>70–80	196 (30.9)	40 (17.5)	57 (20.7)	46 (16.9)
>80–100	93 (14.7)	17 (7.4)	40 (14.5)	19 (7.0)
No. smoking history (%):				
Current	71 (11.1)	38 (16.6)	21 (7.6)	11 (4.0)
Former	284 (44.8)	61 (26.6)	131 (47.5)	84 (30.9)
Never	279 (44.0)	130 (56.8)	124 (44.9)	177 (65.1)
No. hematuria status (%):				
Microhematuria	31 (4.9)	35 (15.3)	104 (37.7)	185 (68.0)
Macrohematuria	603 (95.1)	194 (84.7)	160 (58.0)	82 (30.1)
Unspecified	—	—	12 (4.3)	5 (1.9)

intervals.¹⁹ The external validation of CxbR performance (sensitivity, specificity and NPV) was undertaken in the patients recruited by KP. This data set was also used to evaluate the Cxb test algorithm described in figure 1 and supplementary methods (<https://www.jurology.com>).

RESULTS

CxbR Development and Internal Validation

Patients. The DDS (863) included hematuria evaluation patients from Australia, New Zealand (620)^{10,12} and United States (243; table 2 and supplementary figure 1, <https://www.jurology.com>). Forty patients were diagnosed with LIT and 49 with HIT (table 3 and fig. 2).

Diagnostic Performance. CxbR assigned 96 patients to the “high priority” category, including 56 tumor-positive patients, 47 with HIT and 9 with LIT (fig. 2). In the remaining 767 patients, CxbR assigned 288 to PDP workup and 479 to observation. In the PDP group, 29 patients had UC, of which 2 were HIT and 27 were LIT, while none of the patients assigned to observation had HIT and 4 had LIT (fig. 2).

An additional 5 patients had a confirmed diagnosis of upper tract UC; all 5 patients had been correctly assigned to the high priority CxbR category.

CxbR had a sensitivity (UC) of 95.5% and sensitivity (HIT) of 95.9%, with a NPV of 99.2% for any tumor (table 4). Bootstrap estimates gave a bias-corrected sensitivity (HIT) of 92.4% and specificity (HIT) of 93.8%; bias-corrected sensitivity (UC) was 91.2%, with NPV (UC) of 98.5% (table 4).

External Validation of CxbR Alone and Performance of Algorithm

Patients. Of the 641 consenting KP patients, 559 provided a urine sample, and 548 patients were included in the final analysis (11 patients excluded

due to prior urinary cancer diagnosis; table 2 and supplementary fig. 2, <https://www.jurology.com>). Overall, 242 (44.2%) patients had macrohematuria, 289 (52.7%) had microhematuria, and 17 (3.1%) were not classified. Tumor characteristics are provided in table 3.

External Validation of CxbR Alone. Validating CxbR alone, 29 patients were classified for “high priority” workup (supplementary fig. 3, <https://www.jurology.com>), including 9 tumor-positive patients, all with HIT. In the other 519 patients, CxbR identified 212 needing PDP and 307 for observation. Three patients in the PDP group had UC (2 LIT and 1 HIT), and 2 in the observation group had UC (both LIT; none had HIT). CxbR alone had sensitivity (HIT) of 90.0%, specificity (HIT) of 96.3%, and NPV (HIT) of 99.8% (table 4). All patients with HIT were classified by CxbR as needing either PDP (1) or high-priority workup (9; fig. 3). No patients in the validation data set had a diagnosis of upper tract UC.

Algorithm Performance. In the same data set, we evaluated the performance of the algorithm shown in figure 1. All samples (548) underwent CxbT; 285/548 (52%) had negative results, including 1 patient with a low-

Table 3. Tumor stage and impact in DDS and independent validation data set

Stage	Development Data (863 pts)			Independent Data (548 pts)		
	High Grade	Low Grade	Normal	High Grade	Low Grade	Normal
Normal	—	—	774	—	—	534
Ta	13	40	—	3	4	—
T1	16	3	—	3	1	—
T2	13	—	—	3	—	—
T3	3	—	—	—	—	—
Tis	1	—	—	—	—	—

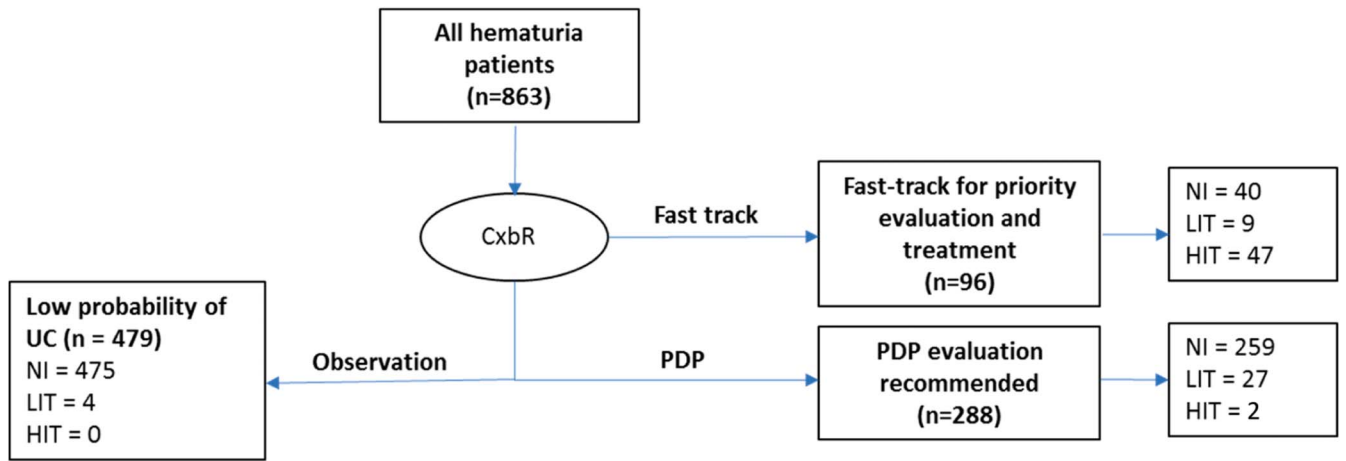


Figure 2. Number of CxbR tests performed, results obtained and number of patients with low or high-impact UC diagnosed within each category in development/internal validation data set. HIT = high-grade Ta, Tis or T1–T3, or concurrent Tis; LIT = low-grade Ta or papillary urothelial neoplasm of low malignant potential. NI, no cancer present.

grade Ta tumor (fig. 3 and supplementary figs. 3 to 5, <https://www.jurology.com>). CxbD was performed on the remaining 263/548 CxbT-positive samples (48%); 164/548 samples (30%) returned a CxbD result indicating a low probability of UC and were ruled out, including 1 patient with a low-grade Ta tumor (fig. 3 and supplementary figs. 3 to 5, <https://www.jurology.com>). Therefore, CxbT followed by CxbD ruled out 449/548 patients (81.9%), but included 2 false-negative results, both low-grade, small Ta tumors. No patients with HIT were incorrectly ruled out.

CxbR was performed on the 99/548 samples (18.1%) not ruled out by CxbT and CxbD, and classified 31/99 patients for observation, 45/99 for PDP workup, and 23/99 for high-priority workup (fig. 3 and supplementary figs. 3 to 5, <https://www.jurology.com>). The concatenated use of CxbT, CxbD and CxbR identified 68 patients (12.4%) as needing either PDP or high-priority workup. When the new AUA guideline risk classification³ was applied to patients in the external validation population (548), 7.7% were classified low-risk, 17.2% as intermediate and 75.2% as high-risk (table 5). Using the AUA guidelines,³ 412/548 high-risk patients would be worked up and all 14 patients (3.4%) with tumors would be correctly identified. Using the Cxb algorithm to identify high-risk patients, 68/548 patients were assigned to workup capturing 11/14 (78.6%) tumors, including all HIT (the remaining 3 being low grade Tas).

Of the 23 patients classified for high-priority evaluation, 9 had HIT. Of the 45 patients classified for PDP evaluation, 1 had LIT, 1 had HIT (high-grade Ta tumor) and 41 did not have UC (table 6). One patient in the observation category also had LIT (fig. 3 and supplementary figs. 3 to 5, <https://www.jurology.com>). The proposed algorithm of Cxb tests accurately ruled out 87% of patients who did not have UC and correctly

identified 100% of HIT patients for further investigation (fig. 3 and supplementary figs. 4 and 5, <https://www.jurology.com>).

DISCUSSION

Bias-corrected estimates in the DDS demonstrated that CxbR alone is highly effective for assigning patients who have HIT to high-priority workup, with 92.4% sensitivity and 93.8% specificity. CxbR has a sensitivity of 91.2% for detecting any tumor and a 98.5% NPV. Data from the KP cohort validated CxbR performance with sensitivity of 90.0% and specificity of 96.3% for HIT. In both the DDS and external CxbR validation studies, all patients with HIT were correctly assigned to PDP or high-priority evaluation.

The reflex algorithm using 3 Cxb tests accurately identified 480/548 (87.6%) patients with low probability of UC to undergo observation (sensitivity 85.7% NPV 99.8%). All patients with positive CxbT

Table 4. Diagnostic performance characteristics of CxbR

	Performance Estimates Observed in DDS (863 pts)		Performance Estimates Observed in Independent Data Set (548 pts)
	HIT*	All Tumors†	HIT*
% Sensitivity	95.9	95.5	90.0
% Specificity	94.0	61.4	96.3
% NPV	—	99.2	—
Bootstrap-adjusted estimates (95% CI):			
% Sensitivity	92.4 (83.3, 96.7)	91.2 (82.2, 96.0)	—
% Specificity	93.8 (86.8, 97.2)	61.0 (41.1, 77.8)	—
% NPV	—	98.5 (97.0, 99.2)	—

* Calculated performance based on proportion of HIT in samples assigned to “high priority” category, ie test positive result is “high priority” and HIT is only true positive outcome.

† Calculated performance based on proportion of any UCs assigned to “high priority” or “PDP”, ie both “high priority” and “PDP” results are test positives, and both LIT and HIT are true positives.

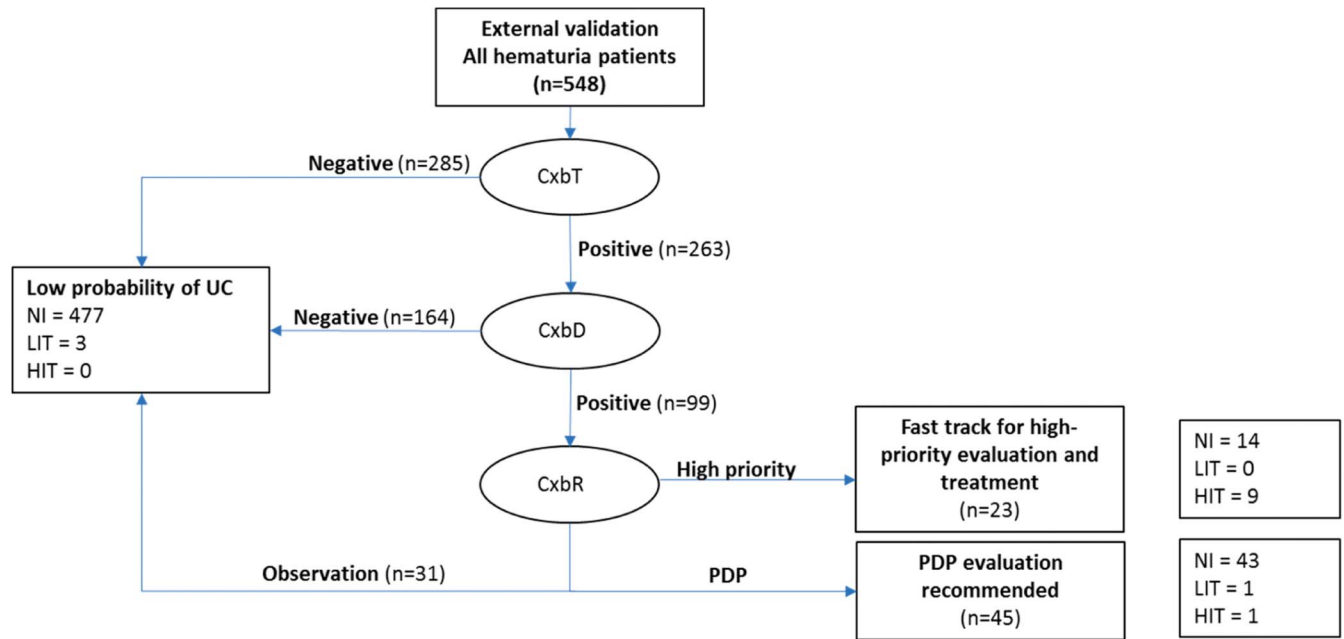


Figure 3. Results of using CxbT, CxbD and CxbR in succession to rule out patients unlikely to have UC, and to identify those who need further workup, in external validation (KP) data set. HIT=high-grade Ta, or T1–T3 or concurrent Tis; LIT=low-grade Ta or papillary urothelial neoplasm of low malignant potential. NI, no cancer present.

and positive CxbD results reflexed to CxbR and all HIT were correctly identified. Three low-grade Ta tumors were missed in this cohort of 480 patients. Therefore, using the 3 tests together on a single urine sample at the initial workup of hematuria provides significant clinical utility to patients, physicians and payers. The tests accurately segregate out patients who need no further workup for bladder cancer from those who have LIT and require PDP, and those who have HIT and should be prioritized for further evaluation.

When the new AUA guideline risk classification³ was applied to patients in the external validation population, 7.7% were classified as low-risk, 17.2% as intermediate and 75.2% as high-risk (table 5), similar to the distribution reported by Wolde et al in a retrospective validation of guideline performance in 15,779 patients.²⁰ Using AUA guidelines to segregate high-risk patients produced a diagnostic yield of 14/412 (3.4%) whereas Cxb had a diagnostic

yield of 11/68 (16.9%), which is 4.8-fold higher. Significant advantages accrue if Cxb is used in the 75.2% patients classified as AUA high-risk since most of these would be reclassified as low-risk using Cxb, enabling physicians to accurately identify those with HIT from this much smaller patient cohort and surveil all others. This approach is consistent with a recent model, which showed that all patients with a positive urine biomarker test should be reclassified as high-risk, irrespective of AUA risk category, and those with a negative result considered as intermediate or low-risk.²¹

Our findings support previous studies showing highly repeatable performance of CxbT and CxbD. All Cxb tests have high sensitivity and NPV,^{9–12} and the combination of CxbT and CxbD has high specificity, sensitivity and NPV.⁶ Most hematuria patients do not have UC and therefore may receive unnecessary expensive and invasive tests, incurring extra costs and time delays.^{22–24} A real-world study

Table 5. Proportion of patients and incidence of cancer in low, intermediate and high-risk categories according to AUA 2020 Guideline and use of 3 sequential Cxb tests

	Proportion of Pts in Each Category (%)			Proportion of Pts with Ca in Each Category (%)		
	Low	Intermediate	High	Low	Intermediate	High
Risk categories defined by AUA 2020 guidelines:						
Wolde et al 2021 (15,779 pts) ²⁰	4.6	11.8	83.6	0.4	1.0	6.3
Independent cohort (548 pts)	7.7	17.2	75.2	0	0	2.7
Risk categories defined by sequential Cxb tests:*						
Independent cohort (548 pts)	87.6	8.2	4.2	0.63	4.4	39.1

* Low-risk= stratified for observation; intermediate-risk= stratified for PDP workup; high-risk= stratified for high-priority workup.

Table 6. Tumors identified in hematuria patients in independent validation set according to segregation into categories using sequence of Cxb tests

Physician Action	Independent Data Set of Hematuria Patients Tested with CxbT, CxbD and CxbR (548 pts)						
	Tumor Stage and Grade						
	Normal	T1 High-Grade	T1 Low-Grade	T2 High-Grade	T2 High-Grade +Tis	Ta High-Grade	Ta Low-Grade
Observation	477	0	0	0	0	0	3
PDP for workup	43	0	0	0	0	1	1
High-priority evaluation	14	3	1	2	1	2	0

showed that using CxbT in the clinical pathway for UC reduced the ordering of cystoscopy by 44% and computerized tomography by 20%.²⁵ CxbT is now routinely combined with imaging at the time of hematuria evaluation in the clinical algorithm used by public health care providers in New Zealand.²⁶ A recently published lookback on the real-world use of that algorithm reported sensitivity of 98.7%, NPV of 99.9%, and specificity of 39%, ruling out the need for further workup in 53% of hematuria patients.²⁷

The current study extends these findings to the utility of consecutively using 3 Cxb tests on hematuria patients. Our results suggest that sequential use of CxbT, CxbD and CxbR on a single urine sample in the initial workup of hematuria correctly identifies all patients with HIT, including all HIT in AUA guideline-defined high-risk patients. Negative results spared 87.6% of individuals, unlikely to have UC, from the need for cystoscopy and imaging. A positive result prioritizes patients for workup, potentially obviating the need for flexible cystoscopy and fast-tracking patients for transurethral resection of the bladder tumor.

For various reasons, many patients who present to primary care with hematuria are not referred for workup. However, the availability of a highly reliable urine test that enriches the diagnostic yield in the hematuria population improves the proportion of at-risk patients being evaluated, resulting in a higher rate of early UC detection in the patients who are referred for workup. Cxb's high level of clinical resolution to rule out patients without UC and prioritize others is likely to reduce referral time and enable specialist resources to be focused on patients most likely to have UC, providing a

clinically meaningful benefit and reducing the cost of care.

Potential limitations of this study are the small proportion of patients with tumors in the validation (KP) data set, with a high proportion of patients with microhematuria selected to represent a more real world cohort than the development data. The development and external validation cohorts had differing proportions of macro and microhematuria and different gender ratios. Further research in larger and more diverse patient groups is needed to verify the current findings. The reflex algorithm missed 3 low-grade Ta tumors; however, as noted by Davidson et al, combining Cxb with imaging increased the NPV to 99.9%.^{26,27}

CONCLUSIONS

CxbR, a noninvasive test with high sensitivity and specificity used on a single urine sample taken at initial investigation, correctly identified all patients with HIT, who can be prioritized for workup. The sequential use of CxbR in combination with CxbT and CxbD was validated to accurately segregate patients with no UC from those with LIT and HIT, allowing those who do not have UC to avoid further workup and focusing resources on HIT patients, with a diagnostic yield 4.8-fold higher than AUA guideline stratification.

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EDITORIAL COMMENTS

Cxbladder measures the expression of 5 genotypic biomarkers (MDK, CDK1, IGFBP5, HOXA13 and CXCR2) in the urine to help identify patients with hematuria at high and low risk for urothelial carcinoma (UC). After development and internal validation in over 850 hematuria patients in 3 separate prospective studies in the U.S., Australia, and New Zealand, Cxbladder Resolve (CxbR) was externally validated on an independent cohort (548) of hematuria patients from a prospective, observational study at Kaiser Permanente in Southern California. Raman et al reported that CxbR had a sensitivity of 95.9% for high-impact bladder tumors (HIT: high grade Ta, Tis or T1–T3) with a negative predictive value (NPV) of 99.2% for any bladder tumor in the development and internal validation cohort. In the external validation cohort, CxbR had a sensitivity of 90.0% for HIT, specificity of 96.3% for HIT, and NPV of 99.8% for HIT.

The above data suggest that urine-based genomic biomarker testing can accurately identify patients with hematuria at high risk of clinically significant bladder cancer and exclude patients at low risk, potentially avoiding invasive

cystoscopy and expensive computerized tomography scan imaging in this cohort (reference 6 in article). Sequential use of Cxbladder testing on a single urine sample could also enrich the diagnostic yield in the hematuria population and more easily identify patients with UC at the primary care level, allowing for potentially earlier detection of disease and improved patient outcomes.

Koya et al also recently evaluated the clinical utility of a new surveillance protocol incorporating Cxbladder testing in real-world practice in patients with prior history of UC at risk for recurrence as an alternative to frequent cystoscopy.¹ This new surveillance protocol reduced the average number of annual cystoscopies by approximately 39% without compromising detection rates with Cxbladder-negative patients safely managed with only 1 cystoscopy per year.

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Optimal diagnostic algorithms for patients with hematuria continue to be refined to improve overall diagnostic yield. Despite the high prevalence of hematuria in the general population, many patients will ultimately have negative invasive testing for urothelial carcinoma that can be both burdensome to patients and costly. For decades, urine-based tumor marker tests have been researched as a potential noninvasive component of the diagnostic paradigm. However, the current AUA microhematuria guidelines discourages the use of urine-based tumor marker testing in the initial evaluation of patients with microhematuria while acknowledging there may be value in specific scenarios (reference 3 in article).

Raman and colleagues report the results of a prospective study that both develops and externally validates the use of Cxbladder Resolve (CxbR), a multiplexed mRNA urinary biomarker test intended for use in patients being evaluated for hematuria. This test combines genomic biomarkers in the urine with phenotypic and clinical data to further risk stratify patients with hematuria. The authors found a high sensitivity (92.4%) and specificity (93.98%) for the CxbR test in the development cohort for

identifying patients with high-impact urothelial tumors. Furthermore, the authors validated the Cxbladder test both alone and in conjunction with a 3-part Cxbladder testing algorithm which identified all high impact tumors with a 99.8% NPV. These data, along with a recently published systematic review on urinary based tumor markers (reference 21 in article), suggest that initial urine based testing in patients with hematuria can likely further refine AUA risk stratification and prioritize high-risk patients for transurethral resection of bladder tumor while minimizing invasive testing in low-risk patients. The present study is an outstanding contribution to the evidence base in urinary biomarker testing for urothelial carcinoma and I look forward to further validation and cost-effectiveness research that will continue to elucidate the best levers to improve diagnostic yields and overall outcomes.

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