Research and Reports in Urology

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Validation of Cxbladder® Triage and Monitor as an Adjunct to Urothelial Carcinoma Diagnosis and Surveillance in a Single Centre

Daniel Magee, Ninan Tharakan, Yuigi Yuiminaga

Department of Urology, Royal Perth Hospital, Perth, WA, Australia

Correspondence: Daniel Magee, Department of Urology, Royal Perth Hospital, Wellington Street, 6000, Perth, WA, Australia, Tel +61 9224 2931, Email danielmagee05@gmail.com

Objective: Bladder cancer is the 10th most common cancer worldwide. The investigation and surveillance commonly involve a combination of upper tract imaging along with visual assessment of the bladder via cystoscopy. This study determined the validity of using Cxbladder® Triage (CxbT) and Cxbladder® Monitor (CxbM) as a suitable adjunct in ruling out urothelial carcinoma (UC) when investigating haematuria or monitoring for recurrence.

Materials and methods: A single centre prospective study where the patients have been referred for investigation of UC or those on routine surveillance of known UC. All patients were counselled with consent obtained prior to midstream urine collection pre-cystoscopy in line with local protocol for urine-analysis to screen for infection with the residual specimen collected for the CxbT or CxbM test. De-identified patient demographic data along with smoking status, risk of environmental exposures, family history, type of hematuria or last date of last recurrence were collected, and the planned cystoscopy would then proceed. The data pertaining to exposure to smoking and type of haematuria are the symptoms and risk factors that are taken into account with CxbT or CxbM to calculate a score, which can then be correlated with the outcome at the end with cystoscopic and imaging investigations.

Results: A combined 236 patients were recruited (CxbT = 134, CxbM = 102) with results showing excellent negative predictive value of 96.43% and 95.16%, respectively. A key result showed that CxbT in combination with upper tract imaging done as routine was able to rule out UC completely in low-risk patients.

Conclusion: We have validated the use of Cxbladder as an adjunct in the investigation and surveillance of UC. It is a non-invasive, accurate and reproducible test that can aid in ruling out UC, specifically for low-risk patients.

Keywords: Cxbladder, urine biomarkers, urothelial carcinoma, bladder cancer

Introduction

Urothelial carcinoma (UC) is the tenth most common malignancy and manifests with visible haematuria or non-visible haematuria.[1,2] Individuals with non-visible haematuria, defined as blood that is only detectable in urinalysis, corresponds to a detection of 1.6%–5% with urothelial bladder cancer, and an estimate of 9% of the individuals have presented with visible haematuria.[2,3] Cystoscopic evaluation of the bladder has been the gold standard for evaluation of bladder malignancy.[2] Additional investigations for haematuria also include imaging with computed tomography (CT), urine biomarkers and urine cytology.[2] There is an increased prevalence of non-visible haematuria in the aging population, with an estimated 20% of the men aged over 60.[3] The investigations undertaken for haematuria are not without risks, such as radiation exposure to computed tomography and potential infections with cystoscopy.[3] Equally, however, delays in diagnosis of urothelial cancer can lead to worse health outcomes.[3]

Validated urinary biomarkers, including UroVysion FISH, NMP22, Bladder EpiCheck, and BTA, have been extensively studied for the detection and surveillance of bladder cancer.[4] UroVysion FISH has been shown to effectively detect chromosomal abnormalities, aiding in recurrence monitoring.[4,5] Bladder EpiCheck, a DNA methylation-based test, has

Received: 11 January 2025
Accepted: 16 March 2025
Published: 18 March 2025

been validated for detecting bladder cancer and monitoring recurrence, demonstrating strong sensitivity and specificity in clinical trials.[6] The current standard of utilising urine cytology has advantages in detecting high-grade tumours and carcinoma in situ, and urine cytology has poor sensitivity in detecting low-grade tumours.[7]

Cxbladder could be an adjunct to the investigation of haematuria to reduce the economic cost, reduce risks from invasive procedure and reduce radiation exposure, as it measures mRNA expression of 5 genotypic biomarkers in samples of urine provided and combined with 4 clinical questions, a score is provided to ascertain the risk of urothelial cancer.[7–9] The clinical questions revolve around age, sex, smoking history, visible haematuria and history of previous UC, combined with the assay to produce a score from 1 to 10.[7–10] With Cxbladder Triage (CxbT) A score of less than 4 with Cxbladder Triage (CxbT) and a score less than 3.5 with Cxbladder Monitor (CxbM) has have low probability of urothelial cancer with a negative predictive value of 98.5% and 97% and sensitivity a sensitivity of 95.1% and 93%, respectively.[8]

The primary aim of the study was to validate the performance of the Cxbladder assay in detecting UC in both newly suspected cases (Triage) and in patients under surveillance (Monitor) for recurrence.

It is important to note that smoking is a well-established risk factor for bladder cancer, with both active and passive exposure significantly increasing the risk. A 2022 meta-analysis found a dose–response relationship between smoking intensity and bladder cancer risk, highlighting that higher consumption correlates with greater risk.[11] Passive smoking is a risk factor that can increase the risk of bladder cancer.[12] Occupational exposure to aromatic amines, such as benzidine and β-naphthylamine, found in industries like dye manufacturing, rubber production, and petrochemicals, has been strongly associated with an increased risk of bladder cancer. A cohort study observed a significant excess of bladder cancer mortality among workers exposed to these chemicals.[13]

Methods

Study Design

This was a single-centre, prospective study. All new referrals to the Urology Department for suspected urothelial cancer (UC), as well as patients under surveillance for confirmed UC, were considered for inclusion in the study from June 2022 till September 2023.

Patient Selection

Patients who were newly referred for suspected UC or were under surveillance to confirm UC were screened for eligibility. Inclusion criteria included patients above the age of 18 able to consent with symptoms or risk factors suggestive of UC or those with a confirmed history of UC. Patients provided informed consent before participating in the study with written information about Cxbladder given to the patient prior to the procedure.

Data Collection

Following consent, patient demographics were recorded, including age and gender, smoking history (current, former >100 cigarettes, or non-smoker), risk of environmental exposure (eg, occupational exposure to carcinogens), type of haematuria (macroscopic, microscopic, none) and previous urothelial carcinoma (date of last recurrence or initial diagnosis). The data recorded was stored on a password secure local Microsoft Excel spreadsheet.

Diagnostic Procedures

Three Diagnostic Modalities Were Utilized in the Study

1. **Urine Collection**: A sterile midstream urine sample was collected from all patients, to assess the risk of a urinary tract infection on the day of, prior to planned cystoscopy. If a suspected infection was present, the patient was postponed with empirical antibiotics provided and the urine sent for microscopy, culture and sensitivity testing. The urine was sent for either CxbT or CxbM assay either as investigation for suspected UC or surveillance of UC.
2. **Flexible Cystoscopy**: Patients underwent a flexible cystoscopy to visually assess the bladder for any suspicious lesions.

3. **Imaging**: Appropriate imaging studies such as computer tomography (CT), intravenous pyelogram or ultrasound kidney ureter, and bladder scans (KUB) were performed based on clinical suspicion either prior or following clinical review and patient presentation to assess the urinary tract for urothelial cancer.

Cxbladder Assay

Urine samples were subjected to the Cxbladder assay, which provided a probability score for the presence or recurrence of UC. Importantly, the results of the Cxbladder assay did not influence clinical decision-making during the study. The treating clinicians were blinded to the assay results, which were reviewed retrospectively and independently after final diagnoses were made using cystoscopy and imaging.

Outcome Measures

The primary outcome measure was the diagnostic accuracy of the Cxbladder assay. Results were compared retrospectively to the final diagnosis determined by a combination of cystoscopy, imaging results or histopathology; if an area of concern was identified during their cystoscopic investigations, the patient proceeded to transurethral resection of bladder tumour (TURBT). Diagnostic performance was assessed by calculating sensitivity, specificity, and negative predictive value (NPV).

Statistical Analysis

Descriptive statistics were used to summarize patient demographics and clinical characteristics. The diagnostic performance of the Cxbladder assay was compared to the final diagnosis. Sensitivity, specificity, PPV, and NPV were reported with 95% confidence intervals. The sample size was sufficient to achieve a 99% power at a 95% significance level.

Ethical Considerations

The study was approved by the local Human Research Ethics Committee (HREC) at East Metropolitan Health Service in Western Australia which is the overarching organization for Royal Perth Hospital and conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007), the CPMP/ICH Note for guidance on Good Clinical Practice and consistent with the principles that have their origin in the Declaration of Helsinki. Compliance with these principles provides assurances that the rights, safety and well-being of the trial participants are respected. Ethics approval number RGS0000004314.

Results

In Table 1, the study highlighted a 1:3.25 (24:78), Female-to-Male ratio for those with previous UC within the CxbM group and a 1:2.35 (40:94) ratio within the CxbT group for those investigated for UC. This ratio is in keeping with well-established sex disparity within the incidence of bladder cancer.[14]

The key results from the study, shown in Tables 2 and 3, demonstrate the diagnostic performance of the Cxbladder assay as a rule out test for UC. The primary focus was on achieving a high negative predictive value (NPV). In both

Table 1 Demographic and Risk Profile of Sample Population

	Overall	Cxbladder Triage	Cxbladder Monitor
Age (years), mean (+- Standard Deviation)	68 ± 16	66 ± 17	72 ± 14
Male, n (%)	172 (72.9)	94 (70.2)	78 (76.5)
Female, n (%)	64 (27.1)	40 (29.8)	24 (23.5)

(*Continued*)

Table 1 (Continued).

	Overall	Cxbladder Triage	Cxbladder Monitor
Smoking status, n (%)			
Never		71 (53)	
>100 Cigarettes in lifetime		63 (47)	
Haematuria			
Never, n *(%)*		6 (4.5)	
Microscopic, n *(%)*		35 (26.1)	
Macroscopic, n *(%)*		93 (69.4)	
Last Diagnosed Urothelial Carcinoma			
Primary			65
Recurrence			37
Highest Previous Stage of Disease			
TaLG			49
TaHG			18
CIS			1
T1HG			24
T1HG + CIS			2
T2HG			5
T2HG + CIS			1
Neuroendocrine Tumour + CIS			1
Other			1

Abbreviation: LG, Low Grade; HG, High Grade; CIS, Carcinoma in-situ.

Table 2 Statistical Results of Cxbladder Triage

Cxbladder Triage	Cx Score <4.0	Cx Score >4.0
Total (134)	55	79
No Malignancy (119)	53	66
Malignancy Proven (15)	2	13
	Value	**95% Confidence Interval**
Sensitivity	86.67%	59.54 to 98.34
Specificity	44.54%	35.43 to 53.93%
Positive Predictive Value	16.46%	13.24% to 20.28%
Negative Predictive Value	96.43%	87.92–99.01%
Accuracy	49.25%	40.52% to 58.02%

Table 3 Statistical Results of Cxbladder Monitor

Cxbladder Monitor	Cx Score <3.5	Cx Score >3.5	Failed Test
Total (102)	62	32	8
No Malignancy (92)	59	26	7
Malignancy Proven (10)	3	6	1
	Value	**95% Confidence Interval**	
Sensitivity	66.67%	29.93% −92.51%	
Specificity	69.41%	58.47–78.95%	
Positive Predictive Value	18.75%	11.62% - 28.82%	
Negative Predictive Value	95.16%	88.54% - 98.04%	
Accuracy	69.15%	58.78% - 78.27%	

CxbT and CxbM groups, the study showed an NPV of 96.4% and 95.16%, respectively, which, although slightly lower than previous multicentre trials with NPVs of 98.5% and 97%, still maintained a high level of reliability and comparability.

Discussion

Adjunct as Rule Out Test for Urothelial Carcinoma

Several studies have evaluated the effectiveness of Cxbladder in clinical settings. A study comparing Cxbladder to urine cytology found that Cxbladder demonstrated a sensitivity of 100%, a negative predictive value (NPV) of 100%, a specificity of 75%, and a positive predictive value (PPV) of 62% in predicting positive cystoscopy findings.[15] These results suggest that Cxbladder is highly effective in ruling out UC, potentially reducing the need for unnecessary cystoscopies. In comparison, urine cytology tends to be more accurate in identifying high-grade urothelial carcinomas but may yield false-negative results for low-grade lesions.[15]

Additionally, research has shown that Cxbladder Monitor significantly outperforms FDA-approved urine tests, including cytology and UroVysion® FISH, in monitoring patients for recurrent UC. The study reported that Cxbladder Monitor had a sensitivity of 91% and an NPV of 96%, surpassing the performance of existing urine-based tests.[9]

Cxbladder has shown to be useful as an adjunct to rule out UC. The high NPV in both CxbT and CxbM is in line with previous multicentre trials conducted with negative predictive value of 98.5% and 97%. Our single centre results were slightly lower than this at 96.4% and 95.16% but comparable and acceptable. Statistical pooling of the results of upper tract imaging with CxbT has shown a slight increase in specificity and positive predictive value and overall accuracy. However, as Cxbladder is a rule-out test, it does compromise and overall reduce the negative predictive value.

Low-Risk Patients

On subset analysis shown in Figure 1, of all the non-smokers within the study for CxbT. Of these, there were 23 patients who were deemed low risk (non-smokers, with microscopic or no haematuria). From these patients, 3 were identified to have superficial bladder cancer, and all were flagged with CxbT. This supports that in this subset of patients they could have avoided a flexible cystoscopy.

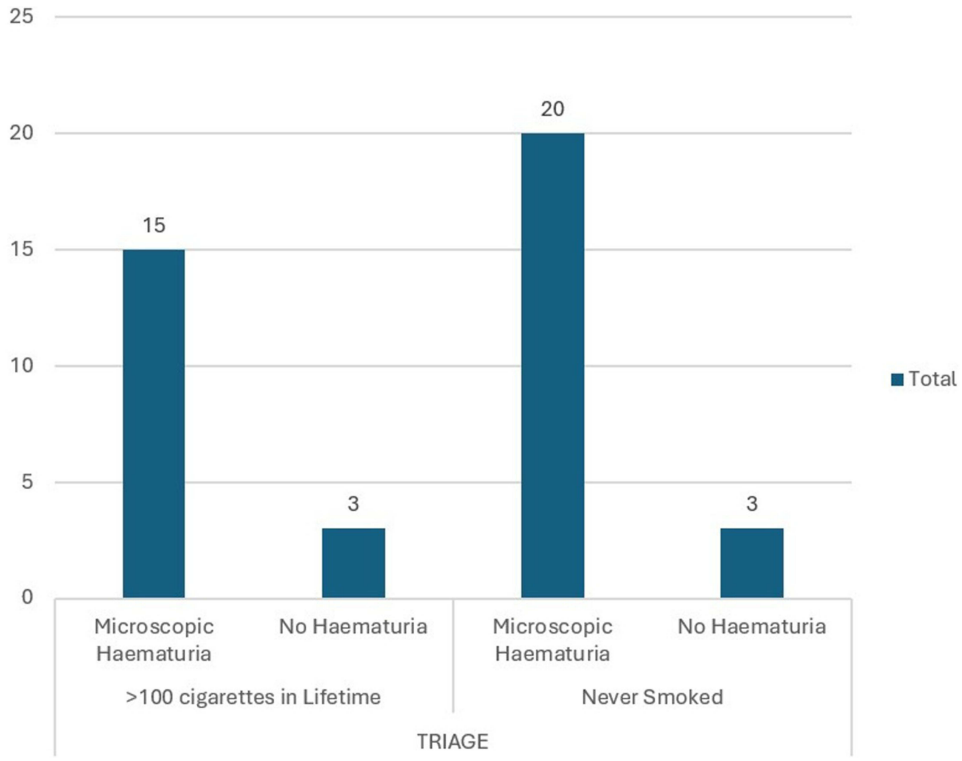

Figure 1 Subset Analysis of Cxbladder Triage low-risk patients (Non-smokers, Microscopic or No Haematuria).

False Negatives

In Table 2, within the CxbT, only two false negatives were captured within our trial, and the final histopathology was superficial non-invasive low grade urothelial carcinoma (TaLG). Concurrent imaging appropriately saw a bladder mass in one of the cases.

Similarly, in Table 3, within the CxbM, we had three false negatives, and all were TaLG. Two of the three cases already had TaLG recurrence previously, and in one case, the highest staging of their previous histopathology was superficial non-invasive high grade urothelial carcinoma (TaHG).

This again supports the idea that Cxbladder is safe and very effective in capturing higher risk cancer.

Limitations

Cxbladder being a specialised urine biomarker that is currently only processed in a laboratory in New Zealand. This can lead to shipping delays which we did experience resulting in a few samples not reaching in a timely manner resulting in them being voided and not included in our analysis. This limitation was overcome by discussions with shipping companies to ensure timely pick-up and delivery.

Conclusion

Within our sample population, we have demonstrated Cxbladder to be a valuable non-invasive adjunctive test for ruling out urothelial carcinoma, particularly in patients at low risk. Our findings reveal high negative predictive values for both Cxbladder Triage and Cxbladder Monitor, aligning with prior multicentre trials and high sensitivity when suspecting urothelial carcinoma.[8–10] The detection rates in our subset analysis of low-risk patients suggest that Cxbladder can accurately identify superficial bladder cancer, potentially enabling these patients to avoid more invasive procedures such as flexible cystoscopy.

The occurrence of false negatives was minimal and exclusively comprised of low-grade, non-invasive urothelial carcinomas (TaLG), which underscores the test's efficacy in detecting higher-risk cancers. Despite logistical challenges associated with the specialised processing of samples in New Zealand, the overall results affirm the safety and effectiveness of Cxbladder. These findings collectively endorse Cxbladder as a reliable, non-invasive diagnostic tool, capable of enhancing patient care by reducing the necessity for invasive procedures while maintaining high standards of cancer detection.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Informed Consent

Each patient was provided with information to read prior to the consent procedure. All physical documents of consent were retained. Patients were advised that consent could be withdrawn at any time and their information would be appropriately removed.

Disclosure

None of the authors has any potential conflict of interest to disclose.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Devlies W, de Jong JJ, Hofmann F, et al. The diagnostic accuracy of cystoscopy for detecting bladder cancer in adults presenting with haematuria: a systematic review from the European Association of Urology Guidelines Office. *Eur Urol Focus*. 2024;10(1):115–122. doi:10.1016/j.euf.2023.08.002
3. Jubber I, Shariat SF, Conroy S, et al. Non-visible haematuria for the detection of bladder, upper tract, and kidney cancer: an updated systematic review and meta-analysis. *Eur Urol*. 2020;77(5):583–598. doi:10.1016/j.eururo.2019.10.010
4. Heard JR, Mitra AP. *Noninvasive Tests for Bladder Cancer Detection and Surveillance: A Systematic Review of Commercially Available Assays. Bl Cancer*. Vol. 10. (Amsterdam, Netherlands), 2024;71–81.
5. Nagai T, Naiki T, Etani T, et al. UroVysion fluorescence in situ hybridization in urothelial carcinoma: a narrative review and future perspectives. *Transl Androl Urol*. 2021;10(4):1908–1917. doi:10.21037/tau-20-1207
6. Fiorentino V, Pizzimenti C, Franchina M, et al. Bladder epicheck test: a novel tool to support urothelial carcinoma diagnosis in urine samples. *Int J mol Sci*. 2023;24(15):12489. doi:10.3390/ijms241512489
7. Laukhtina E, Shim SR, Mori K, et al. Diagnostic accuracy of novel urinary biomarker tests in non-muscle-invasive bladder cancer: a systematic review and network meta-analysis. *Eur Urol Oncol*. 2021;4(6):927–942. doi:10.1016/j.euo.2021.10.003
8. Lotan Y, Daneshmand S, Shore N, et al. A multicenter prospective randomized controlled trial comparing cxbladder triage to cystoscopy in patients with microhematuria: the safe testing of risk for asymptomatic microhematuria trial. *J Urol*. 2024;212(1):41–51. doi:10.1097/JU.0000000000003991
9. Lotan Y, O'Sullivan P, Raman JD, et al. Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. *Urol Oncol Semin Orig Investig*.;358:531.e15–531.e22.
10. Kavalieris L, O'Sullivan P, Frampton C, et al. Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. *J Urol*. 2017;197(6):1419–1426. doi:10.1016/j.juro.2016.12.010
11. Zhao X, Yuanli W, Liang C. Cigarette smoking and risk of bladder cancer: a dose–response meta-analysis. *Int Urol Nephrol*. 2022;54(6):1169–1185. doi:10.1007/s11255-022-03173-w
12. Yan H, Ying Y, Xie H, et al. Secondhand smoking increases bladder cancer risk in nonsmoking population: a meta-analysis. *Cancer Manag Res*. 2018;10:3781–3791. doi:10.2147/CMAR.S175062
13. Pira E, Piolatto G, Negri E, et al. Bladder cancer mortality of workers exposed to aromatic amines: a 58-year follow-up. *J Natl Cancer Inst*. 2010;102(14):1096–1099. doi:10.1093/jnci/djq214
14. Mancini M, Righetto M, Baggio G. Spotlight on gender-specific disparities in bladder cancer. *Urologia*. 2020;87(3):103–114. doi:10.1177/0391560319887327
15. Chai CA, Yeoh WS, Rajandram R, et al. Comparing cxbladder to urine cytology as adjunct to cystoscopy in surveillance of non-muscle invasive bladder cancer-A pilot study. *Front Surg*. 2021;8:659292. doi:10.3389/fsurg.2021.659292