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research article

# The performance of the Xpert Bladder Cancer Monitor Test and voided urinary cytology in the follow-up of urinary bladder tumors

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**Background.** Cystoscopy in complement with urinary cytology represents the gold standard for the follow-up of patients with urinary bladder tumours. Xpert Bladder Cancer Monitor Test (XBC) is a novel mRNA-based urine test for bladder cancer surveillance. The aim of the study was to evaluate the performance of the XBC and voided urinary cytology (VUC) in the follow-up of bladder tumours.

**Patients and methods.** The XBC was performed on stabilized voided urine and VUC was performed on urine samples. The results were compared to cystoscopic findings and histopathological results after transurethral resection of the bladder lesion.

**Results.** For the prediction of malignant histopathological result sensitivity, the specificity and negative predictive value were 76.9%, 97.5% and 93.0% for the XBC and 38.4%, 97.5% and 83.3%, respectively for VUC. For the prediction of suspicious or positive cystoscopic finding sensitivity, the specificity and negative predictive value were 75.0%, 95.2%, and 93.0% respectively for the XBC and 41.7%, 97.6%, and 85.4% for VUC. The sensitivities for papilary urothelial neoplasms of low malignant potential (PUNLMP), low- and high-grade tumours were 0.0%, 66.7% and 100.0% for the XBC and 0.0%, 66.7% and 42.9%, respectively for VUC.

**Conclusions.** The XBC showed significantly higher overall sensitivity and negative predictive value than VUC and could be used to increase the recommended follow-up cystoscopy time intervals. Complementing the XBC and voided urinary cytology does not improve performance in comparison to the XBC alone.

Key words: cystoscopy; Xpert BC Monitor Test; urinary bladder neoplasm; voided urinary cytology

## Introduction

Bladder cancer is the ninth most common cancer worldwide. The estimated age-standardized incidence rate of bladder cancer in Europe in 2012 was 17.7 per 100,000 in men and 3.5 per 100,000 in women.[1] Ninety percent of bladder tumours are urothelial carcinomas of which 80% are classified as a superficial disease at presentation[2], while the rest invade the muscularis propria of the detrusor.

The gold standard for diagnosing and following up bladder tumours is cystoscopy complemented with voided urinary cytology (VUC), and computer tomography urography (CTU), to exclude urothelial carcinoma of the upper urinary tract.[3] Cystoscopy is an invasive and painful procedure with potential short- and long-term complications.

VUC on the other hand is noninvasive, detects urothelial carcinoma in both lower and upper urinary tracts, but its sensitivity for detecting well-differentiated bladder tumours is as low as 16% in contrast to 84% for high-grade tumours[4], therefore VUC cannot replace cystoscopy. The overall sensitivity of CTU for diagnosing bladder cancer is also insufficient to obviate cystoscopy.[5]

In the past two decades, numerous urinary markers for the detection of bladder tumours have been devised, but despite numerous studies, none of these markers have been accepted for diagnosis or follow-up in routine practice or clinical guidelines.[3] Nuclear matrix protein 22 (NMP22), the bladder tumour antigen stat (BTA) test, BTA TRAK test, Immuno Cyt, and fibroblast growth factor receptor 3 (FGFR 3) are FDA approved and commercially available, while many other biomarkers are still being evaluated.[6]

Xpert BC Monitor Test (XBC) is a novel mRNA-based urine test for bladder cancer surveillance, which measures five target mRNAs encoding the following proteins: tyrosine-protein kinase ABL1, corticotropin-releasing hormone (CRH), insulin growth factor 2 (IGF2), uroplakin 1B (UPK1B), and annexin A10 (ANXA10). Except UPK1B, which is a structural protein found in urothelial cells, these proteins play multiple roles during carcinogenesis and are implicated in cell proliferation, division, differentiation, growth, adhesion and signalling. Overall sensitivity (84%) and negative predictive value (93%) of the XBC were reported to be significantly superior to VUC by Pichler *et al*., who published the first study on the XBC in 2017.[7] Since the XBC is noninvasive and requires little additional time and labour for processing of samples in an outpatient setting, it might represent a promising candidate for routine urinary test in follow-up of urinary bladder tumours.

The aims of our study were to compare the performance of XBC and VUC and to determine whether the clinical performance of the XBC is high enough to reduce the need for cystoscopy in follow-ups of urinary bladder tumours.

## Patients and methods

### Patients

The study was approved by The National Medical Ethics Committee of the Republic of Slovenia (0120-566/2018/5) and informed consent was obtained from each participant.

The exclusion criteria were a history of urinary stone disease, ongoing urinary tract infection, suspicion of upper urinary tract tumour, a presence or history of foreign bodies (stent, nephrostomy tube), an invasive procedure on the urinary tract in the past 2 weeks (3 months in case of transurethral resection (TUR) procedure), intravesical chemotherapy or immunotherapy in the past 3 months and prior systemic chemotherapy or radiotherapy of the pelvis.

A total of 54 patients previously diagnosed with bladder tumours were prospectively enrolled in our study from January 2019 to October 2019.

### Detection methods

A single voided urinary specimen was collected before conventional white light cystoscopy during a follow-up visit. The frequency of follow-up cystoscopies was based on the European association of urology (EAU) guidelines.[8] The urine specimen was divided to provide samples for the XBC (4.5ml) and voided urinary cytology (minimally 50 ml). Voided urinary cytology was analysed in the cytopathological laboratory of the Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia, and reported according to the Paris classification system.[9] The results were classified as negative, suspicious, or positive. In the analysis, suspicious results and cellular atypia were included in the positive group for the comparison of detection performance.

In addition to urine cytology, we also analysed urine samples with the Xpert BC Monitor test (Cepheid, Sunnyvale, CA, USA), which measured the levels of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) using reverse transcriptase polymerase chain reaction (RT-PCR) according to the manufacturer's protocol. 4.5 ml of collected urine was transferred to the prepared transport medium within 1 hour after collection and thus stabilized. The sample thus prepared is stable for up to 7 days at temperatures between 2°C and 28°C. The urine thus prepared was transferred to a reagent cartridge in the laboratory, which contained all the reagents needed for RT-PCR analysis. Automated processing included filter capture on cells, lysis of cells by sonication, addition of nucleic acid to dry RT-PCR reagents, transfer to a reaction chamber, RT-PCR multiplexing, and detection. Sample adequacy monitoring (ABL1) ensures that the sample contains human cells and human RNA. An ABL1 signal was required for a valid test result. The internal control detects pattern-related inhibition of

RT-PCR. Control by probe verification (measurement of the fluorescence signal from the probes) is performed prior to sample analysis to monitor bead rehydration, cartridge filling in the cartridge, probe integrity, and toner stability. The Xpert BC Monitor test gives results as "positive" and "negative" based on the results of a linear discriminant analysis (LDA) algorithm that uses the results of the cycle threshold (Ct) of five mRNAs: ABL1, CRH, IGF2, UPK1B and ANXA10 (LDA total calculation). The measurement result is valid if the total LDA value is in the range of -20 to 20. The interpretation of the result is as follows: A "positive" result is obtained if the total LDA is equal to or exceeds the limit value; and ABL1 Ct and total LDA are within the valid range. Not all mRNA targets need be detected for a "positive" test result. A "negative" result is achieved if the LDA Total is below the limit value; and ABL1 Ct is within the valid range. The total LDA of the manufacturer is 0.50 and was determined on the basis of statistical analysis of a large number of samples.[7,10]

The histopathological report on the transurethral resection of a bladder lesion was done by the histopathological laboratory of the Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia. The tumour stage was reported according to the TNM classification of malignant tumours, 8th edition, 2017[11], and the tumour grade was classified according to the 2016 WHO classification.[12] The stage and grade from the histopathological report were used in the analysis of results.

### Statistical analysis

Data was analysed using the SPSS software (Statistical Package for the Social Sciences, version 22.0, IBM Corp., Armonk, NY, USA). After testing for normal distribution using the Shapiro Wilk normality test for the variables, differences in the LDA total were compared using the Mann-Whitney U test for benign and malignant histology groups, negative and positive cistoscopy groups, and negative and positive cytology groups. The optimal cut-off value of the LDA total was determined using the Youden index method, after which the receiver operating characteristic (ROC) curve was calculated to determine the performance of the Xpert BC Monitor test. The diagnostic value of the XBC and VUC was tested by determining the sensitivity (number of true positive tests / sum of number of true positive and false negative tests), specificity (number of true negative tests / sum of true negative and false positive tests) and positive (PPV - number of true positive tests / sum of true positive and false positive tests) and negative (NPV- number of true negative tests / sum of true negative and false negative tests) predictive value. A $p$ value less than 0.05 was considered statistically significant.

## Results

The XBC and VUC were performed in 54 patients scheduled for check-up using conventional white light urethrocystoscopy. Indications for transurethral resection after check-up urethrocystoscopy were: visible recidivant tumour in 12 patients, atypia in VUC in 1 patient, and a missed recidivant tumour that was only discovered during the subsequent check-up urethrocystoscopy. A histology report on the transurethral resection of the bladder lesion was obtained in 12 patients, whereas 2 patients only had electrocoagulation performed on clearly visible malignant recidivant tumours and these two are counted as malignant in further analysis. Out of the 12 histology reports, 11 were malignant and 1 was benign. Patients who had not undergone transurethral resection after the check-up urethrocystoscopy are counted as negative/benign in the histology analysis.

The median LDA totals were 0.2474 (0.1331-0.3617) and 0.8102 (0.4367-1.1836) in the negative and positive cystoscopy groups respectively (Mann-Whitney U = 73.00, $p < 0.000$). The median LDA totals were 0.2486 (0.1286–0.3685) and 0.9869 (0.8872–1.0866) in the negative and positive cytology groups respectively (Mann-Whitney U = 12.00, $p < 0.000$). The Median LDA totals were 0.2467 (0.1357–0.3578) and 0.9273 (0.5904–1.2642) in the negative/benign and malignant histology groups respectively (Mann-Whitney U = 63.00, $p < 0.000$).

Area under the curve in the receiver operating characteristic (ROC) curve for the XBC in predicting cystoscopic finding was 0.855 with a 95% confidence interval between 0.722 and 0.998 (n = 54) (Figure 1).

The area under the curve in the receiver operating characteristic (ROC) curve for the XBC in predicting histology result was 0.882 with a 95% confidence interval between 0.759 and 1.000 (n = 54) (Figure 2).

From the ROC curve for the prediction of cystoscopic findings and histology results, the optimal cut-off value of 0.4923 for the LDA total was determined using the Youden method.

For the prediction of positive cystoscopic findings, the sensitivity and specificity were 75.0% and

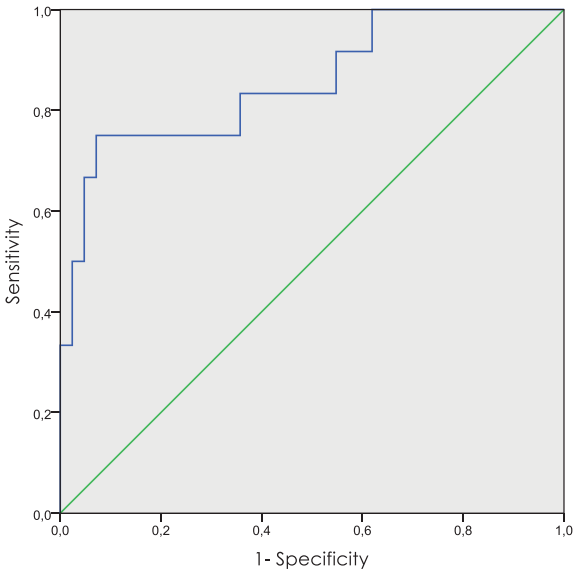


**FIGURE 1.** ROC curve for predicting cystoscopic finding.

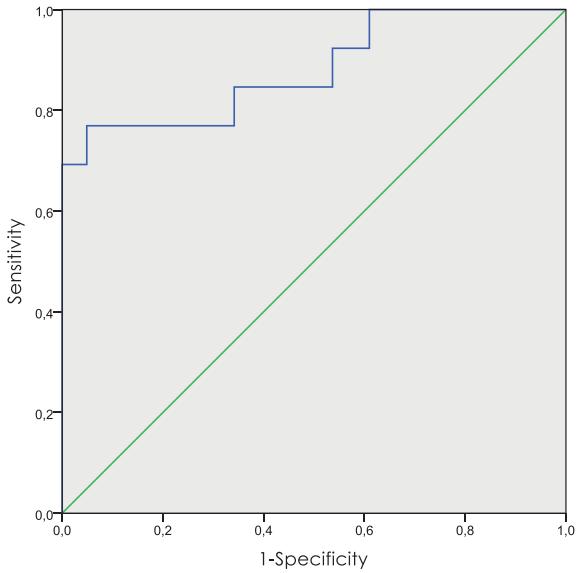


**FIGURE 2.** ROC curve for histology prediction.

95.2% respectively for the XBC (at optimal 0.4923 cut-off value) and 41.7% and 97.6% for VUC. For the prediction of malignant histological results, the sensitivity and specificity were 76.9% and 97.5% respectively for the XBC (at optimal 0.4923 cut-off value) and 38.4% and 97.5% for VUC. Combining the results of the XBC and VUC did not improve the detection of bladder tumours since VUC did not identify any additional patient that was not already positive in the XBC (Tables 2 and 3).

Stratification for grade sensitivities of the XBC were 0.0%, 66.7%, and 100% for papilary urothelial neoplasms of low malignant potential (PUNLMP), low-grade, and high-grade tumours respectively, while the sensitivities of VUC were 0.0%, 66.7%, and 42.9% for the same grade categories respectively. Stratification for stage sensitivities of the XBC were 100%, 80.0%, 100%, and 100% for carcinoma in situ (CIS), Ta, T1 and T2 tumours, respectively, while the sensitivities of VUC were 50.0%, 40.0%, 50.0% and 100% for the same stage categories respectively. (Table 4).

## Discussion

In our study, we have evaluated two voided urinary tests: the XBC and VUC in the follow-ups of urinary bladder tumours. Despite the availability of noninvasive urinary tests, cystoscopy complemented with VUC is still the gold standard for the follow-up of urinary bladder tumours.[3] Urinary cytology has low sensitivity for low-grade tumours, which are the most numerous and represent two-thirds of primarily diagnosed bladder tumours.[13] Consequently, the Paris system for reporting urinary cytology was introduced in 2015, instructing cytopathologists to emphasize that a negative result is only valid for high-grade tumours and "suspicious for high grade urothelial carcinoma" and "atypia" categories are still present in this new classification and are reported based on cell characteristics and numbers.[14] Low grade urothelial neoplasia (LGUN) should only be reported if strict criteria are satisfied, but a suggestion of LGUN can

**TABLE 1.** Summary of the patients' demographics and test results numbers

| Gender | Number of patients | Mean age, years ± SD |
|---|---|---|
| All | 54 (100.0%) | 70.5 ± 8.8 |
| Male | 42 (77.8%) | 70.5 ± 8.7 |
| Female | 12 (22.2%) | 70.5 ± 9.6 |
| **Noninvasive test / standard invasive diagnostic procedure** | **Number of patients with TP / FN / TN / FP noninvasive test result** | |
| XBC / cystocopy (LDATC 0.4923)<br>XBC / cystocopy (LDATC 0.5) | 9 / 3 / 40 / 2<br>8 / 4 / 40 / 2 | |
| VUC / cystoscopy | 5 / 7 / 41 / 1 | |
| XBC / histology (LDATC 0.4923)<br>XBC / histology (LDATC 0.5) | 10 / 3 / 40 / 1<br>9 / 4 / 40 / 1 | |
| VUC / histology | 5 / 8 / 40 / 1 | |

FN = false negative; FP = false positive; LDATC = linear discriminant analysis total cut-off; SD = standard deviation; TN = true negative; TP = true positive; VUC = voided urinary cytology; XBC = Xpert BC Monitor Test

**TABLE 2.** Specificity, sensitivity and positive and negative predictive value of noninvasive urinary tests for the prediction of positive cystoscopic findings

| Test | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| XBC, LDATC 0.4923 | 75.0% | 95.2% | 81,8% | 93.0% |
| XBC, LDATC 0.5 | 66.7% | 95.2% | 80.0% | 90.9% |
| VUC | 41.7% | 97.6% | 83.3% | 85.4% |
| XBC, LDATC 0.4923 + VUC | 75.0% | 95.2% | 81,8% | 93.0% |

LDATC = linear discriminant analysis total cut-off; NPV = negative predictive value; PPV = positive predictive value; VUC = voided urinary cytology; XBC = Xpert BC Monitor Test

**TABLE 3.** Specificity, sensitivity and positive and negative predictive value of noninvasive urinary tests for the prediction of positive histological results

| Test | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| XBC, LDATC 0.4923 | 76.9% | 97.5% | 90,9% | 93.0% |
| XBC, LDATC 0.5 | 69.2% | 97.5% | 90.0% | 90.9% |
| VUC | 38.4% | 97.5% | 83.3% | 83.3% |
| XBC, LDATC 0.4923 + VUC | 76.9% | 97.5% | 90,9% | 93.0% |

LDATC = linear discriminant analysis total cut-off; NPV = negative predictive value; PPV = positive predictive value; VUC = voided urinary cytology; XBC = Xpert BC Monitor Test

be made including recommendation to repeat VUC or perform other diagnostic procedures. The cytological result is affected by the presence of several benign urinary tract conditions and is also dependent on the subjective judgment of the cytopathologist.[15]

Depending on the calculated risk of recurrence and progression, 6 to 15 cystoscopy procedures should be performed in the follow-up after the TUR of superficial bladder tumours within 5 years[3], which represents invasive instrumentation and a risk for complications for patients, as well as healthcare and economic burden.

Replacing cystoscopy with a noninvasive, simple, low-cost urine-based test with sufficient performance could modify these follow-up schedules. Such a noninvasive test should have high sensitivity to detect small recurrent high-grade tumours and avoid the growth and progression to a muscle-infiltrative disease, which considerably aggravates the patient's prognosis and quality of life.[16]

The expression levels of the markers combined in linear discriminate analysis (LDA) are used to classify samples as negative or positive using a linear model.[10] While the LDA total value primarily serves as a criterion for a positive result of the XBC at the cut-off value suggested by the manufacturer (0.5), we used the ROC curve and Youden method to optimize the cut-off value to 0.4923, which improved the sensitivity of XBC for the prediction of positive cystoscopic findings from 66.7% to 75.0%. Considering the LDA total value rather than reporting the results of the XBC as negative/positive could also be used in longitudinal studies as was shown by Hurle *et al*., who monitored the LDA total in the active surveillance of patients with non-muscle-invasive bladder cancer in the Bladder Cancer Italian Active Surveillance project (BIAS) and reported that an increasing LDA total in a patient is connected to progressing bladder tumours and could be used to initiate follow-up cystoscopy instead of enforcing firm scheduling protocols. They also proposed that in this setting, the LDA total cut-off would be 0.4.[17]

Our data shows that sensitivities of the XBC for predicting positive or suspicious cystoscopic results is considerably higher than for VUC (75.0% *vs*. 41.2% respectively), and also NPV is improved in comparison to VUC (93.0% *vs*. 85.4% respectively). The results are comparable to the results of the studies of Van Valenberg *et al*. and Pichler *et al*. (74 % and 84% for sensitivity and 93% and 93% for NPV respectively).[7,18] In contrast, the study performed by Elia *et al*. reported an overall sensitivity of only 46.2% for the XBC, though the vast majority (87%) of the detected bladder tumours in that study were low-grade.[19] In our study, none of the patients with negative XBC had a positive VUC, therefore complementing the results of the XBC

**TABLE 4.** Sensitivity of the XBC and voided urinary cytology stratified by tumour stage and grade. Number of cases detected by noninvasive test against number of cases detected by hystology analysis is shown in the parenthsis

| Test / Stage | CIS | Ta | T1 | T2 | Grade | PUNLMP | low grade | high grade |
|---|---|---|---|---|---|---|---|---|
| XBC, LDATC 0.4923 | 100% (2/2) | 80.0% (4/5) | 100% (2/2) | 100% (1/1) | | 0.0% (0/1) | 66.7% (2/3) | 100.0% (7/7) |
| VUC | 50.0% (1/2) | 40.0% (2/5) | 50.0% (1/2) | 100% (1/1) | | 0.0% (0/1) | 66.7% (2/3) | 42.9% (3/7) |

LDATC = linear discriminant analysis total cut-off; PUNLMP = papillary urinary neoplasm of low malignant potential; VUC = voided urinary cytology; XBC = Xpert BC Monitor Test

with VUC did not increase sensitivity, specificity, NPV, or PPV, which was also observed in the study by Pichler *et al*.[7]

Until more studies on the XBC have been conducted, the range of sensitivities and NPVs cannot be estimated, though our data and already published studies[7,18] show that the performance of the XBC is at least comparable to previously studied bladder cancer biomarkers for which studies have reported wide variability in performance. A recent review of bladder cancer biomarkers summarized data from several studies listing the sensitivities of NMP22 in the range of 24% to 81%, of BTA STAT 40% to 72%, of ImmunoCyt 50% to 85%, and of UroVysion 13 to 100%. Probably due to the variability in performance, the authors of this review conclude that current commercially available urinary biomarker-based tests are not sufficiently validated to be widely used in clinical practice.[20]

Cystoscopy was negative in two of our patients, who were positive in the XBC. One of them had an atypical VUC and a high-grade T1 tumour was confirmed with transurethral resection biopsy (TURB), while in another patient with negative VUC, a tumour was identified at a follow-up cystoscopy after 3 months and a high-grade T1 tumour was confirmed. Both cases suggest that even though cystoscopy is the gold standard for the follow-up of bladder tumours, it should be complemented with a noninvasive urinary test.

The high discrimination power of the XBC between malignant and benign histology groups is a consequence of the highly significant difference between the median LDA total in both groups (0.2486 for benign and 0.9869 for malignant group respectively). Overall sensitivity and NPV for the prediction of a malignant histological result of the XBC (76.9% and 93.0%) greatly outperforms the sensitivity and NPV of VUC (38.4% and 83.3)

Stratification for the tumour grade sensitivity of the XBC was comparable to VUC for PUNLMP (0%) and low-grade tumours (66.7%), though the sensitivity of VUC for high-grade tumours was only 42.6%, while no high-grade tumour was missed by the XBC. Improved performance was also reported by Pichler *et al*.[7], who showed considerably higher sensitivity for low-grade tumours (77% *vs*. 3%) and high-grade tumour +s (100% *vs*. 25%) for the XBC and VUC respectively. However, the study published by Elia *et al*. showed significantly lower sensitivity for low-grade (42%) and high-grade tumours (85.7%).[19] When tumours are stratified by grade, the sensitivity in our study is comparable to the study of Van Velenberg *et al*.[18] in categories T1 and T2 (100%) and to the study of Pichler *et al*.[7] in categories CIS (100%) and Ta (80%).

Since high-grade bladder tumours have a stage progression risk of 23% in five years compared to only 4% in low-grade tumours[21], the XBC could emerge as a noninvasive test to guide the follow-up schedule for the cystoscopic surveillance of a bladder tumour, as was already proposed by Hurle *et al*.[17] Small, Ta low-grade papillary recurrence, which the XBC could miss, does not present an immediate danger to the patient and early detection is not essential for successful therapy,[3,22] while for T1 and T2 high-grade tumours or CIS, the XBC is very likely to be positive.

Even though XBC has high sensitivity and NPV for high grade tumours, the rate of adoption of this and other noninvasive molecular tests to routine clinical practice will also depend on financial resources of the healthcare system. Molecular tests typically cost three to five times the price of VUC (XBC 250 EUR and VUC 40 EUR in Slovenia) and are more expensive than single rigid or even flexible cystoscopic procedure in countries, where fees of healthcare professionals are low to moderate.

The main limitation of the present study is the low number of included patients (54) and therefore the low number of patients with recurrent tumours identified at follow-up (14 patients or 25.9%), which is a consequence of the low recurrence rate and study design. A similar proportion of new recurrence tumours (30.7% ) was also observed in a larger study by Pichler *et al*.[7], while the largest published study only reported a 17.9% recurrence rate.[18] Furthermore, only 1 patient in the negative histology group had a TUR done, while all the others were counted as negative on the basis of the negative cystoscopic result, possibly missing a small hidden lesion or a lesion in the upper urinary tract.

## Conclusions

The XBC showed significantly higher overall sensitivity and negative predictive value than VUC. Its ability to detect intermediate and high-risk superficial tumour recurrences could modify follow-up cystoscopy schedules by increasing the recommended time intervals in patients with a negative XBC. Complementing the XBC and voided urinary cytology does not improve the performance in comparison to the XBC alone.

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