Diagnostic Accuracy of MCM5 for the Detection of Recurrence in Nonmuscle Invasive Bladder Cancer Followup: A Blinded, Prospective Cohort, Multicenter European Study

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Purpose: Detection of MCM5 containing cells in urine has been shown to be indicative of the presence of a bladder tumor on primary diagnosis. In this study we evaluate diagnostic performance of ADXBLADDER in patients undergoing cystoscopic surveillance in nonmuscle invasive bladder cancer followup.

Materials and Methods: A multicenter prospective blinded study was performed at 21 European centers with patients undergoing cystoscopy for nonmuscle invasive bladder cancer surveillance, diagnosed in the preceding 2 years. Urine was collected from all eligible patients and ADXBLADDER-MCM5 testing was performed. Performance characteristics were calculated by comparing MCM5 results to the outcome of cystoscopy plus pathological assessment.

Results: Of 1,431 eligible patients enrolled 127 were diagnosed with a bladder cancer recurrence. The overall sensitivity for the ADXBLADDER-MCM5 test in detecting bladder cancer recurrence was 44.9% (95% CI 36.1–54) with a 75.6% sensitivity for nonpTaLG tumors (95% CI 59.7–87.6). Specificity was 71.1% (95% CI 68.5–73.5). The overall negative predictive value was 93% (95% CI 91.2–94.5). However, ADXBLADDER was able to rule out the presence of a nonpTaLG recurrent tumor with a negative predictive value of 99.0% (95% CI 98.2–99.5). No statistically significant differences in the performance of ADXBLADDER were observed as a result of age or sex.

Conclusions: This large blinded prospective study demonstrates that in the followup of patients with nonmuscle invasive bladder cancer ADXBLADDER is able to exclude the presence of the most aggressive tumors with a negative predictive value of 99%. These results indicate that ADXBLADDER could be incorporated in the followup strategy of nonmuscle invasive bladder cancer.

Abbreviations and Acronyms

BCa = bladder cancer
CIS = carcinoma in situ
HG = high grade
LG = low grade
MCM5 = minichromosome maintenance protein 5
NMIBC = nonmuscle invasive bladder cancer
NPV = negative predictive value
TURBT = transurethral bladder tumor resection

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Bladder cancer is the tenth most common cancer worldwide with an estimated 549,000 cases diagnosed and 200,000 people dying of the disease in 2018.[1] It also has the highest recurrence rate of all cancers, with up to 78% of cases recurring, making it a major health care

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challenge and expensive to treat.[2] Currently bladder cancer followup relies upon repeat flexible cystoscopies at regular intervals, the frequency of which is determined by risk categorization of the patient. Many patients at low risk for progression would benefit from a reduction in the number of cystoscopies performed. However, to achieve this, a test with a high NPV would be required to ensure cancers at higher risk for progression are not missed.[3] While numerous urinary biomarkers have been developed with this aim, none have been successfully implemented in clinical practice due to poor performance and a lack of high quality prospective validation studies.[4]

MCM5, a DNA licensing factor, is a biomarker of proliferation, which has previously been established as an excellent biomarker in BCa diagnosis.[5] All cells capable of proliferation express MCM5, and in normal urothelium MCM5 expression is restricted to cells within the basal proliferative compartment. The cells lining a normal bladder and shed into urine are therefore MCM5 negative. In the case of urothelial carcinomas, whereby cells grow uncontrollably, MCM5 expression is found throughout all layers of the urothelium, resulting in cells exfoliated from the surface of the bladder that express MCM5. The presence of these MCM5 positive cells in the urine sediment is indicative of a tumor. Detection of MCM5 in urine sediment has been found to be an excellent biomarker for bladder cancer, with strong sensitivity and a very high NPV in previous studies.[5–7] ADXBLADDER (Arquer Diagnostics, Sunderland) is a commercially available MCM5-enzyme-linked immunosorbent assay able to detect MCM5 in urine sediment in patients with BCa with hematuria.[7] Our purpose was to evaluate the diagnostic performance of ADXBLADDER in a large, blinded, prospective, multicenter European study of patients undergoing cystoscopic surveillance in BCa followup.

MATERIALS AND METHODS

Study Design

This was a commercially sponsored, cross-sectional, prospective, blinded study carried out at 21 urology centers in Europe. Overall 1,718 patients were enrolled between August 2017 and July 2019, ethical approval was received from all local Research Ethics Committees (REC Reference: 17/NE/0174) and informed consent obtained from all patients before collection of urine samples. All eligible patients were recruited from urology centers across 21 European study centers (supplementary table 1, https://www.jurology.com). Patients recruited were older than 18 years, with a previous (pathologically confirmed) diagnosis of nonmuscle invasive bladder cancer in the preceding 24 months, undergoing cystoscopic surveillance at the urology clinic. Patients able to understand the study, provide informed consent, and capable of providing a full void urine of greater than 10 ml were considered eligible. Collected data included age at the time of cystoscopy, stage, grade and followup. Any patients with known active calculi or prostatitis were excluded from the study. Any urological instrumentation to the urinary tract in the preceding 14 days also rendered patients ineligible.

Patients were considered to be bladder cancer recurrence positive where a lesion detected on cystoscopy was confirmed to be a recurrent bladder tumor pathologically at TURBT/biopsy. Other cystoscopic findings such as inflammation or erythema were considered negative, unless a biopsy was indicated and was pathologically determined as positive for a recurrent bladder tumor. Where a lesion was detected by cystoscopy but no biopsy/TURBT carried out (for example if patients received fulguration or undertook a watchful waiting approach) patients were excluded from analysis. Any patient with no cystoscopy result was excluded from final analysis.

Urine Collection and Processing

Full void urine was collected before cystoscopy and processed within 48 hours of collection. Ten to 50 ml of full void urine were centrifuged for 5 minutes at 1,500 g at room temperature. Urine sediment pellets were resuspended in ADXBLADDER lysis buffer. Lysates were incubated for 30 minutes at room temperature to allow complete lysis before being stored below -20C until testing.

MCM5 Testing

To determine MCM5 status (MCM5 positive/MCM5 negative) urine sediment lysates were subjected to ADXBLADDER testing as per manufacturer instructions. All clinicians were blinded to results of ADXBLADDER testing and laboratory staff performing ADXBLADDER were blinded to the results of cystoscopy. Briefly 100 μl of lysate was added to ADXBLADDER microtiter plates and incubated for 60 minutes at room temperature, a wash step was carried out prior to the addition and 30-minute incubation of 100 μl ADXBLADDER-Conjugate. A second wash step was performed before incubation for 30 minutes with 100 μl TMB (3,3',5,5'-tetramethylbenzidine), the reaction was stopped by addition of ADXBLADDER-STOP solution. Optical density was measured at 450 and 630 nm. Any sample with optical density greater than or equal to the predefined cutoff as per manufacturer instructions was considered to be MCM5 positive. Samples with optical density below this were considered to be MCM5 negative.

Statistical Analysis

A sample size calculation was performed and it was calculated that a total estimated sample size of 752 to 1,504 was required to ensure that the maximum marginal error of estimate did not exceed from 10% with 95% confidence level based upon the previously reported sensitivity of ADXBLADDER[7] and an assumed prevalence of 5% to 10%. Sensitivity and specificity were calculated using ADXBLADDER results compared to the reference of cystoscopy plus pathological assessment. Comparisons of diagnostic accuracy were calculated based upon the AUC. All statistical analyses were performed with STATA®

(version 12.1) and statistical significance was indicated if p values were less than 0.05.

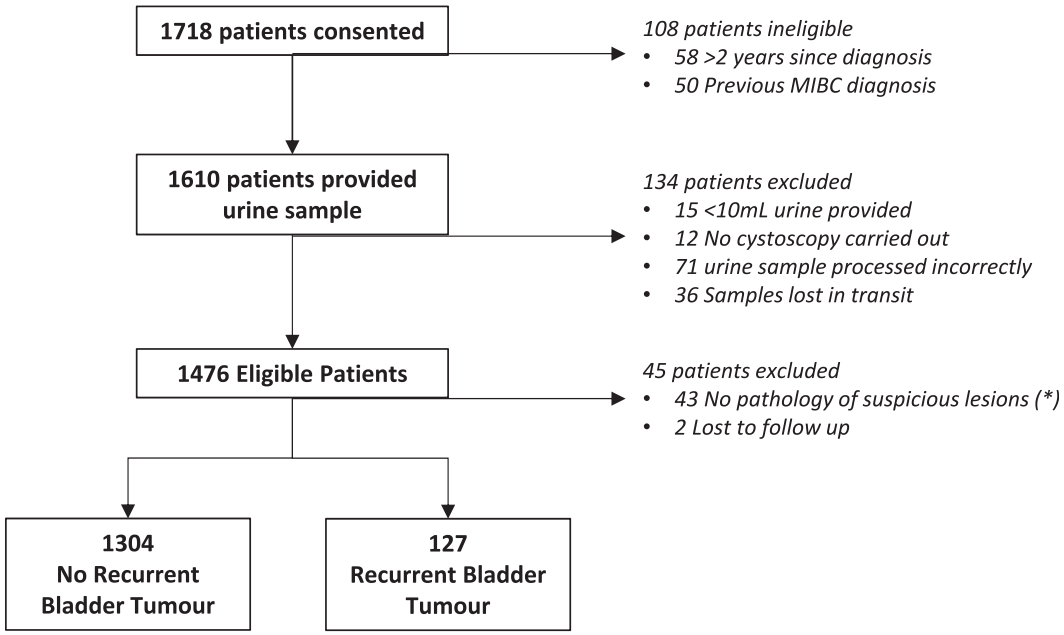
RESULTS

Between August 2017 and July 2019, 1,718 patients attending the urology clinic for cystoscopic surveillance in BCa followup gave informed consent and provided urine samples. Median patient age was 73 years (IQR 66–80), with 1,276 (76.3%) males and 442 females (25.7%). Overall 108 (6.3%) patients were subsequently found to have had a previous diagnosis of muscle invasive bladder cancer or more than 2 years since a positive TURBT/biopsy and, therefore, were excluded as per the protocol. Of the remaining 1,610 patients 71 (4.4%) were excluded due to improper urine sample processing (incorrect volume of lysis buffer added to urine sediment pellet/inadequate storage of urine before processing), 36 (2.2%) were lost in transit by courier, 15 (0.9%) failed to provide 10 ml urine and 12 (0.7%) did not proceed with cystoscopy. In addition, 2 (0.1%) patients were lost to followup and 43 (2.7%) were diagnosed with a recurrent BCa but had no pathological examination of suspicious lesions (where lesion was removed by fulguration, or patient entered active surveillance) and, therefore, were excluded from analysis (see figure).

Of the remaining 1,431 eligible patients 127 were diagnosed with a pathologically confirmed recurrent BCa (prevalence 8.9%, 95% CI 7.5–10.5). Median age of the population was 73 years (IQR 66–80), with 1,062 males (74.2%) and 369 females (25.8%). The majority of the patients (51.6%) had previously been diagnosed with a low grade pTa tumor and most were between 12 and 24 months of a positive TURBT (81.8%). For 459 patients (32.1%) the last treatment received was TURBT, while 534 (37.3%) patients had received bacillus Calmette-Guérin instillations and 424 (29.6%) had received intravesical chemotherapy (of which 403 were mitomycin C based). All baseline demographic and clinical characteristics of the participants are shown in table 1.

Overall 57 of the 127 BCa recurrence positive samples tested positive for MCM5 (overall sensitivity 44.9%, 95% CI 36.1–54) while 927 of the 1,304 BCa recurrence negative samples were negative for MCM5, yielding a specificity of 71.1% (95% CI 68.5–73.5) and an area under the ROC curve of 0.57 (95% CI 0.51–0.62) (table 2, supplementary fig. 1, *A*, https://www.jurology.com). Of the 127 cancers 41 were nonpTaLG tumors, with MCM5 testing positive in 31 of these patients, demonstrating a sensitivity of 75.6% (95% CI 59.7–87.6) with an area under the ROC curve of. 0.71 (95% CI 0.62–0.80) (supplementary fig. 1, *B*, https://www.jurology.com). Overall NPV of MCM5 testing was 93.0% (95% CI 91.2–94.5). However, MCM5 was able to rule out a nonpTaLG recurrent tumor with a NPV of 99.0% (95% CI 98.2–99.5) (table 3, supplementary fig. 2, https://www.jurology.com).[8]

Interestingly, MCM5 sensitivity in patients with a previous diagnosis of CIS or HG (88.9%, 95% CI 51.8–99.7 and 63.3%, 95% CI 43.9–80.1, respectively) was superior to that in patients with a previous LG diagnosis (34.8%, 95% CI 25.0–45.7) (supplementary



Standards for Reporting of Diagnostic Accuracy (STARD) diagram of patient recruitment and enrollment. Asterisk indicates patients received fulguration or undertook a watchful waiting approach. *MIBC*, muscle invasive bladder cancer.

Table 1. *Patient demographics at time of recruitment*

	Total Population (1,431)	Bladder Ca Recurrence Pos (127)	Bladder Ca Recurrence Neg (1,304)
No. sex (%):			
Male	1,062 (74.2)	94 (74.0)	968 (74.2)
Female	369 (25.8)	33 (26.0)	336 (25.8)
Median age (IQR)	73 (66–80)	73 (68–80)	73 (66–80)
Stage + grade of last TURBT/biopsy (%):			
Ta low grade	738 (51.6)	86 (67.7)	652 (50.0)
Ta high grade	376 (26.3)	20 (15.7)	356 (27.3)
T1	267 (18.7)	16 (12.6)	251 (19.2)
All CIS*	145 (10.1)	9 (7.1)	136 (10.4)
No. mos between TURBT + ADXBLADDER test (%):			
Less than 3	19 (1.3)	3 (2.4)	16 (1.2)
3–12	242 (16.9)	34 (26.8)	208 (16.0)
Greater than 12	1,170 (81.8)	90 (70.9)	1,080 (82.8)
No. last treatment received (%):			
bacillus Calmette-Guérin	534 (37.3)	25 (19.7)	509 (39.0)
Intravesical chemotherapy:	424 (29.6)	37 (29.1)	387 (29.7)
Mitomycin-C	377 (26.3)	33 (26)	344 (26.4)
Mitomycin-C + hyperthermia	23 (1.6)	1 (0.7)	22 (1.7)
Mitomycin C + nephrourectomy	3 (0.2)	-	3 (0.2)
Synergo	11 (0.8)	1 (0.7)	10 (0.8)
Epirubicin	7 (0.5)	1 (0.7)	6 (0.5)
Doxyrubicin	1 (0.07)	-	1 (0.08)
Gemcitibin	1 (0.07)	-	1 (0.08)
GemRIS	1 (0.07)	1 (0.7)	-
None (TURBT only)	459 (32.1)	64 (50.4)	395 (30.3)
Other	14 (1.0)	1 (0.7)	13 (1.0)

* Includes patients with CIS alone and CIS with co-occurring papillary tumors. Of the 145 cases only 23 had CIS alone (20 bladder cancer negative, 3 bladder cancer positive).

fig. 3, https://www.jurology.com). No significant difference in diagnostic performance of MCM5 was observed as measured by the area under the ROC curves stratified according to sex or age (supplementary figs. 4 and 5, https://www.jurology.com, respectively).

Overall 43 patients were excluded from final analysis as there was no pathological confirmation of suspicious lesions identified on cystoscopy, either because lesions were removed by fulguration or a watchful waiting approach was adopted (see figure). Analysis of these excluded patients reveals that if they were to be considered as positive for BCa recurrence, the sensitivity in patients with nonpathologically confirmed tumors is identical to that of pTaLG tumors (sensitivity 30.2%, 95% CI 17.2–46.1 for nonpathologically confirmed tumors vs 30.2%, 95% CI 20.8–41.1 for pTaLG) (supplementary table 2, https://www.jurology.com). Overall this would have demonstrated 170 patients as positive for recurrent bladder tumors (prevalence 11.5%, 95% CI 10.0–13.3) with 70 samples testing positive for MCM5 giving an overall sensitivity of 41.2% (95% CI 33.7–49.0), specificity of 71.1% (95% CI 68.5–73.5) and overall negative predictive value of 90.3% (95% CI 89.1–91.4) (supplementary table 3, https://www.jurology.com).

DISCUSSION

Current diagnostic tools for the detection of recurrent BCa tumors in followup include cystoscopy and cytology for patients with high risk NMIBC, as recommended by guidelines. Despite urine tests being known to have a positive impact on quality of followup cystoscopy,[9] to date they have failed to be used in clinical practice due to a lack of high quality prospective studies reflecting clinical practice and poor performance/diagnostic accuracy.[3] Here we present one of the largest prospective studies of a urinary biomarker in BCa followup to date, carried out at 21 high volume centers across Europe.

This blinded prospective study demonstrated that in followup of patients with NMIBC, ADXBLADDER, a MCM5 test, was able to exclude the presence of the most aggressive tumors (nonpTaLG) with a NPV of 99%. While there was a very high number of cancer negative cases, the large prospective nature of this study is reflective of daily clinical practice.

MCM5 detection is dependent upon the presence of MCM5 containing cells in the urine. Smaller lower grade tumors shed fewer cells into the urine than larger high grade tumors,[10] which may explain the difference in sensitivity of the MCM5 test in

Table 2. *2 x 2 Contingency table*

All Tumors			NonpTaLG Tumors		
	Bladder Ca Recurrence			Bladder Ca Recurrence	
ADXBLADDER	Pos	Neg	ADXBLADDER	Pos	Neg
Pos	57	377	Pos	31	403
Neg	70	927	Neg	10	987

Table 3. *Performance characteristics of ADXBLADDER by tumor type*

Tumor Type (No.)	% Sensitivity (95% CI)	% NPV (95% CI)
All tumors (127)	44.9 (36.1—54)	93 (91.2—94.5)
Stage:		
pTa (107)	38.3 (29.1—48.2)	93.4 (91.7—94.8)
pT1 (12)	75 (42.8—94.5)	99.7 (99.1—99.9)
pT2 (2)	100 (15.8—100)	100 (99.6—100)
All CIS (14)*	71.4 (41.9—91.6)	99.6 (99—99.9)
Grade (2004):		
LG (86)	30.2 (20.8—41.1)	94 (92.3—95.4)
HG (37)	73 (55.9—86.2)	99.0 (98.2—99.5)
Grade (1973):		
G1 (26)	42.3 (23.4—63.1)	98.5 (97.9—98.9)
G2 (78)	35.9 (25.3—47.6)	95.0 (94.1—95.7)
G3 (19)	73.7 (48.8—90.9)	99.5 (98.9—99.8)
Tumor No.:		
Solitary (61)	45.9 (33.1—59.2)	96.6 (95.7—97.3)
Multiple (57)	42.1 (29.1—55.9)	96.6 (95.7—97.2)
Tumor size (cm):		
Less than 1 (79)	36.7 (26.1—48.3)	94.9 (94—95.7)
1—3 (23)	65.2 (42.7—83.6)	99.1 (98.5—99.5)
Greater than 3 (1)	Not applicable	Not applicable
pTaLG (86)	30.2 (20.8—41.1)	94.0 (92.3—95.4)
nonpTaLG (41)	75.6 (59.7—87.6)	99.0 (98.2—99.5)

* Ten of the CIS cases had co-occurring papillary lesions, 4 cases had CIS alone.

detecting recurrent tumors compared to the diagnostic indication (45% recurrence BCa vs 73% primary BCa), with most recurrent tumors being smaller and low grade.[11] Although the sensitivity of the MCM5 test for recurrent pTaLG tumors was lower than that observed for primary tumors,[7] small, recurrent, low grade tumors are slow growing and pose minimal risk of progression, with an average of only 2% for low risk NMIBC cases progressing within 10 years.[12] Indeed, evidence suggests that active surveillance in these patients is a safe and cost-effective approach to managing these tumors.[13,14] Therefore, the observed sensitivity for detecting asymptomatic low risk recurrent NMIBC with a MCM5 test, which will be detected at the next followup cystoscopy, would provide a safe and cost-effective strategy in the BCa followup pathway.

EORTC (European Organisation for Research and Treatment of Cancer) risk stratification is of the utmost importance in daily clinical practice, notably the proficiency with which MCM5 detection was able to identify intermediate and high risk was much higher, with a 75.6% sensitivity and a negative predictive value of 99%. Currently many urologists perform more regular cystoscopy than is recommended in guidelines, particularly in low risk disease, as a precautionary measure.[15] ADXBLADDER with its 99% NPV for high risk recurrence would provide reassurance to these urologists, with a negative test providing reassurance that an aggressive tumor can be ruled out, thereby safely reducing the frequency of cystoscopy and decreasing costs. Furthermore, the prospective nature of this large multicenter study ensures that the observed prevalences closely reflect the situation in most urology clinics, signifying that a MCM5 test such as ADXBLADDER could be effectively incorporated in the followup strategy of NMIBC.

At present there is no consensus for managing high risk NMIBC with an abnormal urine test but a negative cystoscopy. Given the risk of progression in these patients, we suggest that high risk patients with an abnormal ADXBLADDER test result should continue to have another cystoscopy as per the guidelines. In those with a negative ADXBLADDER result the followup regimen and the rhythm of surveillance could be adapted, sparing unnecessary cystoscopies for patients at low risk for progression, with a negative ADXBLADDER result having ruled out a high risk tumor.

Recently many new biomarkers have been developed, with reported overall sensitivities higher than that demonstrated in this study. However, the prevalence of HG disease in these studies is very high, impacting positively upon the overall sensitivity. Importantly to this study the ability of a negative MCM5 test to rule out high grade tumors is very similar to that of all of the available new tests with similar sensitivities for high grade disease for MCM5 (75.6%) and the other biomarker tests such as Xpert BC Monitor (83%),[16] Bladder Epicheck (88.9%)[17]and CxBladder (97%).[18] Further independent validations are required as the studies are relatively small with few centers performing the tests.

The limitations of the reported study are that the false-positive rate was higher than that of cytology. In addition, as there were no followup data, there could be no indication if false-positives of ADXBLADDER were early detection of a subclinical recurrence that could not be detected by cystoscopy, as has been reported with other urinary biomarkers such as UroVysion.[19] Furthermore, no further investigations were performed in patients with negative cystoscopy but positive for ADXBLADDER and, therefore, the presence of upper tract tumors cannot be ruled out. While the study population is very large and confidence intervals are narrow for most of the reported performance characteristics, the nonpTaLG sensitivity is based on 41 recurrences. Therefore, the CIs are slightly wider, although very much in line with previously reported data for MCM5.[7,20] Further multicenter validation of ADXBLADDER on larger numbers of patients is warranted, ideally with a health economic component to truly demonstrate cost-effectiveness.

It is clear that the suitability of a test such as ADXBLADDER in routine clinical practice is well adapted, due to its noninvasive nature, with no constraint for patients. The exceptionally high NPV for high risk NMIBC recurrences demonstrated here may lead to a reduced need for repeat cystoscopies, with the potential of distinguishing patients

with high risk of disease recurrence from those with no further disease following primary BCa diagnosis.

CONCLUSION

This large blinded prospective study demonstrates that in the followup of patients with NMIBC a negative ADXBLADDER test result can exclude the presence of the most aggressive tumors with a NPV of 99%. These results indicate that ADXBLADDER could be incorporated in the followup strategy of NMIBC as a rule-out test.

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