



## Original Article

# Comparison of the performances of the ADXBLADDER test and urinary cytology in the follow-up of non-muscle-invasive bladder cancer: a blinded prospective multicentric study

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## Objective

To compare directly the performance of the ADXBLADDER test with that of cytology in the detection of non-muscle-invasive bladder cancer (NMIBC) recurrences.

## Background

ADXBLADDER is a urine test based on the detection of MCM5, a DNA licensing factor expressed in all cells capable of dividing. Expression is usually restricted to the basal stem cell compartment; however, in malignancy, MCM5-expressing cells can be found throughout the epithelium. Detection of MCM5 in urine sediment can be indicative of the presence of a bladder tumour.

## Patients and Methods

A multicentre prospective, blinded study was carried out from August 2017 and July 2019 at 21 European Union centres, 14 of which collected matching cytology data. Urine was collected from patients prior to cystoscopy. Urine cytology and ADXBLADDER were performed and compared to the diagnosis obtained by cystoscopy. The performance of cytology and ADXBLADDER were then compared.

## Results

The overall performance of ADXBLADDER demonstrated a sensitivity of 51.9%, a specificity of 66.4%, and a negative predictive value (NPV) of 92%. The sensitivity of ADXBLADDER for low- and high-grade recurrences was 44.1% and 58.8%, respectively. By contrast, cytology sensitivity was 16.7%, specificity was 98% and NPV was 90.7%. Cytology sensitivity for both low- and high-grade disease was 17.6%.

## Conclusions

ADXBLADDER detection of both low- and high-grade NMIBC recurrence is superior to that of cytology, with ADXBLADDER able to exclude the presence of high-grade recurrence in 97.8% of cases compared to 97.1% with cytology. These results show that ADXBLADDER has promise as a more reliable alternative to urine cytology in the follow-up of NMIBC.

## Keywords

biomarker, bladder cancer, follow-up, cytology MCM5, non-muscle-invasive bladder cancer, surveillance, #uroonc, #BladderCancer, #blcsm, #utuc

## Introduction

The cytological analysis of urine sediments has been used as a tool to diagnose urinary tract malignancies for over 70 years [1]. Urine cytology in conjunction with cystoscopy is considered to be the 'gold standard' and is recommended in the European Guidelines for high-risk non-muscle-invasive bladder cancer (NMIBC). The excellent specificity of urine cytology is well documented; however, the reported sensitivity has been extremely variable [2]. Whilst, historically, cytology was thought to have good sensitivity for high-grade disease, with reported sensitivities of between 38% and 84% [3], recently, larger multicentric studies have demonstrated much lower sensitivities, with a prospective study of 1016 patients demonstrating an overall sensitivity of 22%, 13% for low-grade tumours, 23% for high-grade tumours and 25% for high-risk tumours [4,5]. One of the potential reasons for the variability in the sensitivity for cytology is that the technique is highly subjective, requiring a skilled uro-pathologist for interpretation, so adds variability, particularly in multicentric studies. Despite a number of classification systems being brought into practice, most recently the Paris System for reporting Urine Cytology (Paris system) [6], the complication of equivocal results (such as those reported as atypical), has resulted in the use of cytology in follow-up becoming increasingly questionable. There is therefore a growing need for alternative non-invasive diagnostic tools in bladder cancer (BCa).

The field of urinary biomarker research is a very active area of research, with the focus being on developing non-invasive and cost-effective strategies to aid in the diagnosis of BCa. However, despite this, no urinary biomarker has been successfully implemented into clinical practice due to low sensitivities and specificities and a lack of high-quality prospective studies to support their use in clinical practice [7].

The method of detecting MCM5 in urine sediment has been shown to accurately diagnose BCa in a number of previous studies, with particularly high sensitivity for high-risk disease (high-grade, pT1 and above and carcinoma *in situ* [CIS] tumours) [8–11].

In the present study, we report results from a large, prospective, European multicentre study, conducted with the objective of assessing the performance of ADXBLEADDER compared to that of cytology in the detection of NMIBC recurrence.

## Patients and Methods

### Study Population

Between August 2017 and July 2019, 1718 patients were enrolled prospectively to the study. Ethical approval was obtained locally at all sites (REC reference: 17/NE/0174), and informed consent was sought from all patients. Patients

considered eligible for the study were those attending the urology clinic for a BCa follow-up cystoscopy, with a previous diagnosis of pathologically confirmed BCa in the preceding 24 months. Patients had to be over 18 years of age, able to understand the study and give informed consent, and capable of providing a full void urine sample of greater than 10 mL. Patients unable to provide 10 mL of urine, and those who had urological instrumentation less than 2 weeks prior to their appointment, or had active calculi or prostatitis were excluded from the study.

All patients were required to undergo flexible cystoscopy as part of their BCa follow-up, the results of which were recorded. If the cystoscopy was found to be normal the patients were considered to be BCa recurrence-negative. If a lesion was detected on cystoscopy and was pathologically determined to be positive at biopsy/transurethral resection of bladder tumours (TURBT), the patient was considered to be BCa recurrence-positive. Other findings on cystoscopy (i.e. inflammation or erythema) were considered to be BCa recurrence-negative unless a biopsy was clinically indicated which was pathologically determined to be positive. Cases in which a lesion was detected but no pathological assessment was carried out, were excluded from final analysis (e.g. if the detected lesion was removed by diathermy, or the patient underwent watchful waiting).

Cytology testing was carried out locally in a subset of patients and cases classified as per the Paris system were included [6]. For the purposes of this study, a positive cytology result was recorded for those reported as 'suspicious for high-grade urothelial carcinoma', 'low-grade urothelial neoplasia' or 'high-grade urothelial carcinoma', whilst those reported as 'atypical', 'negative for high-grade urothelial carcinoma' and 'non-diagnostic' were considered to have a negative cytology result.

### Urine Collection and Processing

Each patient provided a full void urine sample of at least 10 mL, which was collected prior to their cystoscopy and processed within 48 h of collection. Sample processing involved centrifuging the samples at room temperature for 5 min at 1500 g. The resulting sediment pellet was then resuspended in ADXBLEADDER lysis buffer (10 µL lysis buffer per 1 mL urine) for 30 min at room temperature to allow adequate lysis of cells, after which the lysate samples were stored at  $\leq -20^{\circ}\text{C}$  until MCM5 testing was required.

### MCM5 Testing: ADXBLEADDER

MCM5 is a protein essential for DNA replication present in all cells capable of dividing but lost in terminally differentiated cells. In normal urothelium MCM5-expressing cells are confined to the basal proliferative compartment, however, in the presence of urothelial carcinoma,

MCM5-positive cells are present throughout the urothelium and exfoliated into the urine. ADXBLADDER is a diagnostic test that detects MCM5 in urine sediment. Patient samples were tested for the presence of MCM5 using ADXBLADDER (Arquer Diagnostics Ltd, Sunderland, UK) as per the manufacturer's instructions. All laboratory staff performing the ADXBLADDER test were blinded to the results of the cystoscopy and cytology, and clinicians were blinded to the ADXBLADDER results. Briefly, two 100- $\mu$ L replicates of each lysate sample and controls were added to the ELISA microtitre plate and incubated at room temperature for 60 min with shaking (700–1200 rpm). A wash step was performed, followed by a 30 min incubation of 100  $\mu$ L of anti-MCM5 horseradish peroxidase conjugated antibody at room temperature. Following a second wash step, 100  $\mu$ L 3,3',5,5'-Tetramethylbenzidine was added to the plate and incubated for 30 min at room temperature before ADXBLADDER stop solution was added to terminate the colorimetric reaction. Sample optical density was then measured at 450 and 630 nm and normalized to the control values. Samples with a normalized optical density greater than or equal to the assay threshold, as per the manufacturer's instructions, were considered positive for MCM5, and samples with an optical density below this were regarded as MCM5-negative.

### Statistical Analysis

Comparisons of diagnostic accuracy were calculated based on the area under the receiver-operating characteristic curve (AUC). Significance for ADXBLADDER vs cytology sensitivities was calculated using McNemar's exact test. All statistical analyses were performed with STATA 12.1. Statistical significance was indicated if *P* values were < 0.05.

## Results

Patients attending the urology clinic for NMIBC follow-up cystoscopy were recruited between August 2017 and July 2019. Patients provided informed consent and supplied a urine sample. Of the 1718 patients recruited, 514 had matched cytology data, 11 of these patients had a bladder lesion detected but no associated pathology of the tumour and were therefore excluded from analysis. Therefore, 503 patients with results for both ADXBLADDER and cytology were included in the comparative analysis of performance characteristics for the two tests (Fig. 1). Of the remaining 503 eligible patients recruited with matching cytology data 373 were men and 130 women, with a median (interquartile range) age of 72 (65–77) years. Fifty-four of the patients were diagnosed with a pathologically confirmed recurrent BCa tumour (11% prevalence, 95% CI 8.2–13.8).

Of the 503 patients, 203 (40%) had a previous diagnosis of a low-grade pTa tumour, whilst the remaining patients had a

previous diagnosis of high-grade pTa, CIS or pT1. A total of 99% of the patients underwent their previous TURBT more than 3 months previously, with 49% undergoing TURBT 3–12 months previously and the remaining 50% 12–24 months previously. For 22% of the population, TURBT was the last treatment received; however, the majority had received BCG after their last TURBT (57%), the remaining 21% had received another form of intravesical chemotherapy (primarily mitomycin C [18%]). All patient demographics are provided in Table 1.

In the detection of recurrent bladder tumours, ADXBLADDER outperformed cytology's sensitivity for all tumour types. The overall performance in this cohort of matching cytology patients demonstrated that ADXBLADDER was able to correctly identify a BCa recurrence in 28 of the 54 BCa recurrence-positive patients, yielding a sensitivity of 51.9% (95% CI 37.8–65.7%), whilst 298 BCa recurrence-negative patients were correctly identified by ADXBLADDER, giving a specificity of 66.4% (95% CI 61.8–70.7), and a negative predictive value (NPV) of 92% (95% CI 88.5–94.7; Table 2).

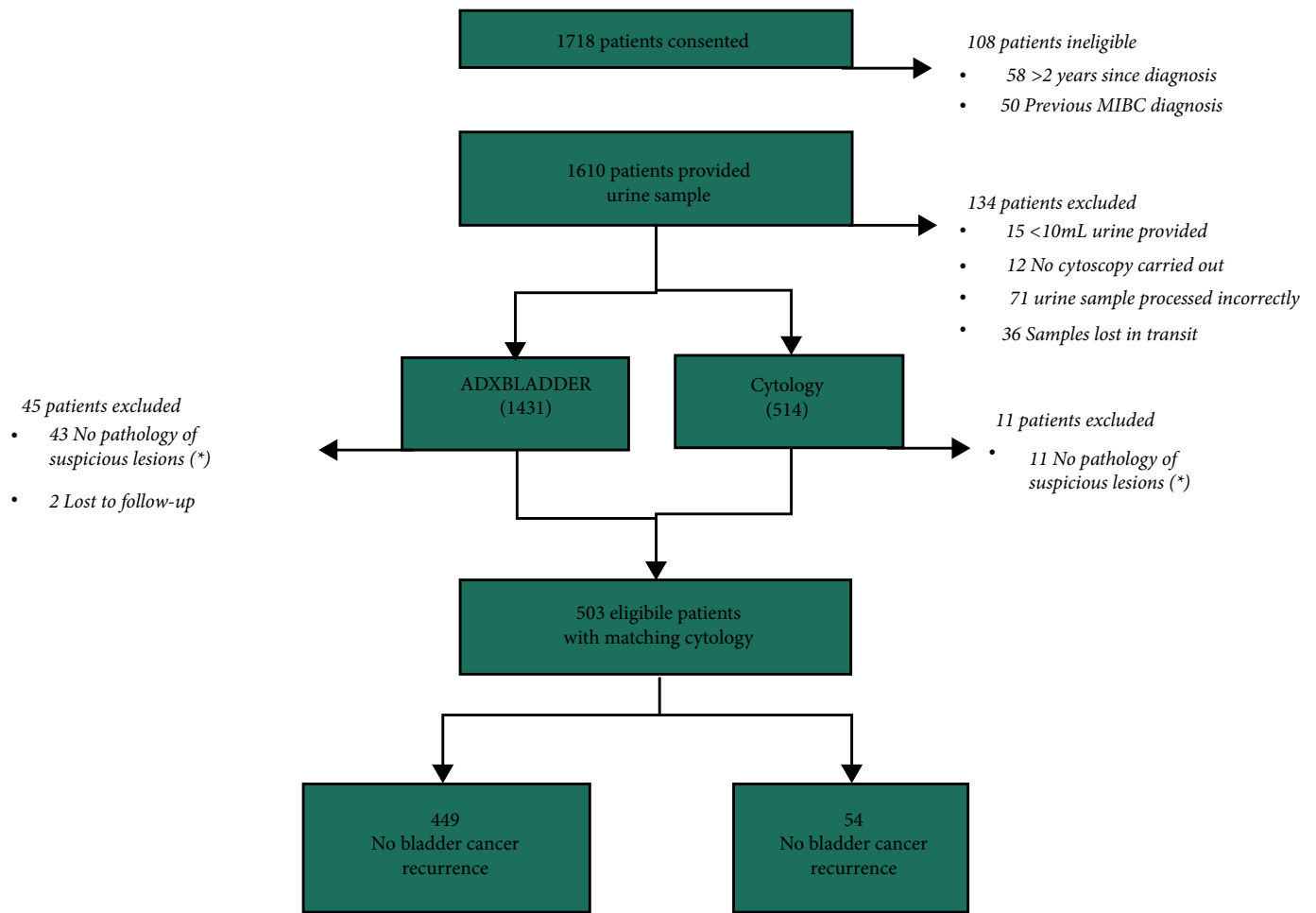
In contrast, the ability of cytology to correctly identify BCa recurrences was significantly poorer, with cytology only detecting nine out of the 54 BCa-positive recurrences, yielding a sensitivity of 16.7% (95% CI 7.9–29.3), specificity remained high with only 9/449 BCa-negative patients giving a positive cytology result, thereby generating a specificity of 98% (95% CI 96.2–99.1) and an overall NPV of 90.7% (95% CI 87.8–93.2; Table 3).

The sensitivity of ADXBLADDER was consistently higher than that of cytology regardless of the recurrent tumour type. For low-grade, CIS and high-grade recurrences the sensitivity of ADXBLADDER was 44.1% (95% CI 27.2–62.1), 60.0% (95% CI 26.2–87.8) and 58.8% (95% CI 32.9–81.6), respectively, and 65% (95% CI 40.8–84.6) for non-pTa low-grade tumours, whilst for cytology the sensitivity for both low- and high-grade recurrences was 17.6% (95% CI 6.8–34.5) and 30% (95% CI 6.7–65.3) for CIS, (Table 4, Fig. S2) and 15% (95% CI 3.2–37.9) for non-pTa low-grade tumours. Whilst there was no significant difference in the AUC, as a result of the very high specificity of cytology (Fig. S1), the increased sensitivity of ADXBLADDER in comparison with cytology was statistically significant for all tumours (*P* < 0.001), Low-grade tumours (*P* = 0.02), high-grade tumours (*P* = 0.04) and high-risk (non-pTa low-grade) tumours (*P* = 0.006; Fig. 2).

## Discussion

European Association of Urology guidelines currently recommend cytology in the follow-up of NMIBC for high-risk patients as an adjunct to cystoscopy, as white-light

**Fig. 1** Standards for Reporting of Diagnostic Accuracy (STARD) patient recruitment and enrolment outline. MIBC, carcinoma invading bladder muscle.



cystoscopy alone is not considered to be sufficient [12]. However, concerns over the poor sensitivity of cytology to detect recurrent tumours at high risk of progression have highlighted an unmet need within the follow-up of NMIBC for a non-invasive, cost-effective adjunctive test to cystoscopy.

In the present study, we establish that ADXBLADDER is significantly more sensitive than cytology at detecting recurrent bladder tumours for all tumour sub-type recurrences ( $P < 0.001$ ), detecting at least twice the number of recurrences as cytology for low-grade, high-grade, CIS and non-pTa low-grade tumours. The overall sensitivity of cytology in this study of 16.7% (17.6% for high-grade disease, 15% for non-pTa low-grade tumours) is consistent with the previously reported sensitivity of cytology in another large multicentric study [5], but is lower than other previous reports [13]. In contrast, ADXBLADDER demonstrated a much higher sensitivity of 51.9% (58.8% for high-grade disease and 65% for non-pTa low-grade tumours).

Whilst cytology requires the time of a skilled uro-pathologist, ADXBLADDER is a simple ELISA requiring no interpretation

(an optical density reading of greater than the predefined cut-off being classed as positive), in addition the results from an ADXBLADDER test can be available within 3 h.

Furthermore, using the currently recommended Paris system for classifying cytology, a non-negligible number of samples in this study were categorized as non-diagnostic (14.9%), and a further 4.2% were classified as atypical. This latter subgroup was also regarded as negative in the present study since, in spite of absence of a true consensus on the optimal management of an atypical cytology result, it is generally accepted that it should be followed up akin to the ‘negative’ category [4]. In contrast, ADXBLADDER gave non-equivocal results for all samples.

As a result of the study being blinded, one of the limitations is that there was no further testing carried out in patients with a positive ADXBLADDER test, but negative cystoscopy and cytology; therefore, the presence of upper tract tumours cannot be excluded in these patients. Additionally, there was no follow-up available for this subset of patients to determine if there may have been a

**Table 1** Patient demographics at recruitment.

Characteristic	Total population (N = 503)	BCa recurrence-positive (n = 54)
Men	373 (74)	45 (83)
Women	130 (26)	9 (17)
Age, years	72 (65–77)	73 (67–79)
<b>Stage and grade of last TURBT/biopsy, n (%)</b>		
Ta low-grade	203 (40)	28 (52)
Ta high-grade	143 (28)	12 (22)
T1	136 (27)	12 (22)
CIS; all*	63 (13)	5 (9)
CIS: alone*	7 (1)	1 (2)
<b>EAU risk group</b>		
Low	72 (14)	9 (17)
Intermediate	112 (22)	18 (33)
High	300 (60)	26 (48)
Undetermined	19 (4)	1 (2)
<b>Time between TURBT and ADXBLADDER test, n (%)</b>		
<3 months	4 (0.8)	–
3–12 months	245 (49)	28 (52)
>12 months	254 (50)	26 (48)
<b>Last treatment received, n (%)</b>		
BCG	288 (57)	19 (35)
Intravesical chemotherapy	105 (21)	12 (22)
Mitomycin-C	91 (18)	9 (17)
Mitomycin-C + hyperthermia	1 (0.2)	–
Synergo	7 (1)	1 (2)
Epirubicin	2 (0.4)	1 (2)
Doxorubicin	1 (0.2)	–
GemRIS	1 (0.2)	1 (2)
None (TURBT only)	110 (22)	23 (43)

*BCa, bladder cancer; CIS, carcinoma in situ; EAU, European Association of Urology; TURBT, transurethral resection of bladder tumour. \*Seven patients had CIS alone, 56 patients had co-occurring papillary lesions.*

**Table 2** Two × two contingency table for ADXBLADDER.

ADXBLADDER	BCa recurrence-positive	BCa recurrence-negative	Total
Positive	28	151	179
Negative	26	298	324
Total	54	449	503

*BCa, bladder cancer.*

**Table 3** Two × two contingency table for cytology.

Cytology	BCa recurrence-positive	BCa recurrence-negative	Total
Positive (high-grade UC/suspicious for high-grade UC and low-grade UN)	9	9	18
Negative (negative for high-grade UC, atypia and non-diagnostic)	45	440	485
Total	54	449	503

*BCa, bladder cancer; UC, urothelial carcinoma; UN, urothelial neoplasia.*

sub-clinical recurrence, which was undetected by the other tests, which has been demonstrated with other biomarker tests [14]. Another limitation of the study is that all cytology was reviewed locally; therefore, the multicentric nature of the study may have introduced variability in the cytology reporting, which may explain the low sensitivity observed in comparison with some previous reports.

The lack of *a priori* power analysis sample size calculation is also a potential limitation of the study. These calculations were provided only for the overall cohort of 1718 patients, of which the current 514 cases with matched cytology data represent a sub-study. However, using the McNemar two-sided test for paired comparisons, it is calculated that 31 patients with a recurrence are required to detect the observed difference in sensitivity, (51.9% vs 16.7%), to be significant at  $\alpha = 0.05$  and power = 0.80. In all, 54 patients had a recurrence, which provides a *post hoc* power of 97% to detect the observed difference to be significant at  $\alpha = 0.05$ .

While the overall sensitivity of ADXBLADDER is similar to that reported by other commercially available urinary biomarkers, such as NMP22, BTA Stat and UBC Rapid, there was a relatively high prevalence of pTa low-grade recurrent tumours within this population. In high-grade or late-stage disease, the sensitivity of ADXBLADDER (65%) is higher than that previously observed by these markers (31% for NMP22 ELISA, 16% for NMP22 BladderChek) [5].

Although the observed specificity of cytology was higher than that of ADXBLADDER, the role of ADXBLADDER as a test must be considered [15]. ADXBLADDER significantly outperformed urine cytology in the detection of both low- and high-grade BCa recurrences. Hence, it should be considered as a more reliable alternative to urine cytology, when indicated by current guidelines, as an adjunct to cystoscopy.

The present results clearly demonstrate that ADXBLADDER has a superior sensitivity when compared to cytology, for the detection of recurrent bladder tumours, revealing a promising case for ADXBLADDER to replace cytology in the follow-up of NMIBC.

## Conflicts of Interest

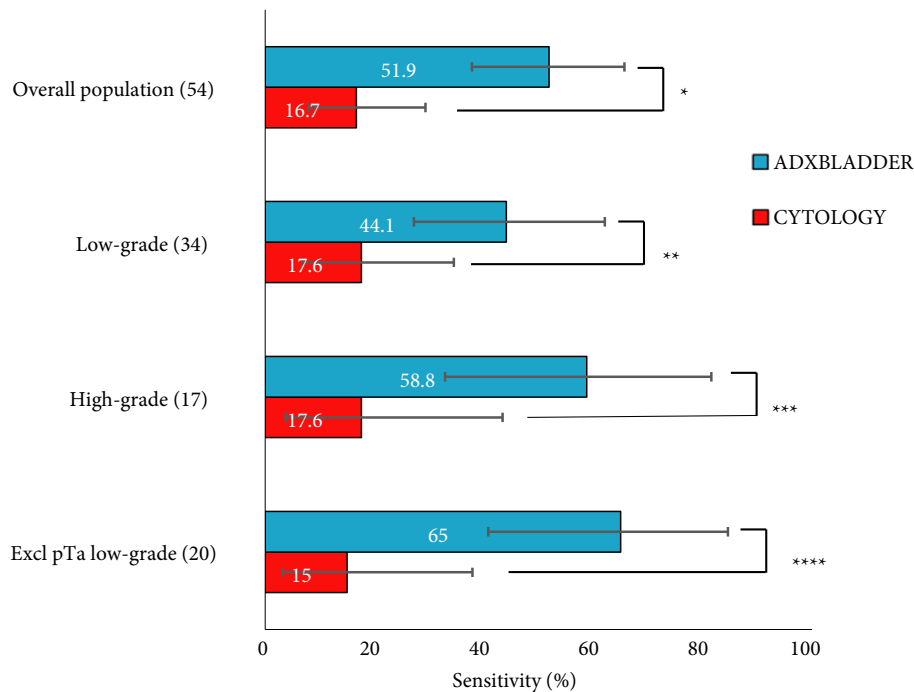
This was a commercially sponsored study by Arquer Diagnostics Ltd. M. Roupret, P. Gontero, S. R. C. McCracken, T. Dudderidge, M. Colombel, F. Longo, E. Montanari and J. Palou are paid consultants to Arquer Diagnostics. J. A. Witjes has received fees for lecture honorarium for Nucleix. None has any shareholding in Arquer Diagnostics or any related company. J. Stockley and

**Table 4** Comparison of performance characteristics of ADXBLADDER with cytology by recurrent tumour classification.

Type of tumour	ADXBLADDER		Cytology	
	Sensitivity, % (95% CI)	NPV, % (95% CI)	Sensitivity, % (95% CI)	NPV, % (95% CI)
All tumours (n = 54)	51.9 (37.8–65.7)	92.0 (88.5–94.7)	16.7 (7.9–29.3)	90.7 (87.8–93.2)
<b>Stage</b>				
pTa (n = 42)	45.2 (29.8–61.3)	92.9 (89.5–95.4)	19 (8.6–34.1)	93 (90.3–95.1)
pT1 (n = 8)	75 (34.9–96.8)	99.4 (97.8–99.9)	12.5 (0.3–52.7)	98.6 (97–99.4)
CIS: all* (n = 10)	60 (26.2–87.8)	98.8 (97.4–99.4)	30 (6.7–65.2)	98.6 (97–99.4)
CIS: alone* (n = 3)	100 (29.2–100.0)	100 (n/a)	0 (0–70.8)	99.4 (99.37–99.39)
<b>Grade</b>				
Low (n = 34)	44.1 (27.2–62.1)	94.1 (91–96.4)	17.6 (6.8–34.5)	94.2 (91.8–96.1)
High (n = 17)	58.8 (32.9–81.6)	97.8 (95.6–99.1)	17.6 (3.8–43.4)	97.1 (95.2–98.4)
<b>Number of tumours</b>				
Solitary (n = 29)	44.8 (26.5–64.3)	95.1 (93.2–96.4)	17.2 (5.9–35.8)	95.1 (94.2–95.8)
Multiple (n = 21)	52.4 (29.8–74.3)	96.9 (95.2–98.0)	19.1 (5.5–41.9)	96.5 (95.7–97.1)
<b>Size of tumours</b>				
<1 cm (n = 37)	43.2 (27.1–60.5)	93.5 (91.5–95.1)	18.9 (8–35.2)	93.8 (92.8–94.7)
1–3 cm (n = 11)	63.6 (30.8–89.1)	98.8 (97.3–99.4)	9.1 (0.2–41.3)	97.9 (97.5–98.3)
Low-grade pTa (n = 34)	44.1 (27.2–62.1)	94.1 (91–96.4)	17.6 (6.8–34.5)	94.2 (91.8–96.1)
Non-low-grade pTa (n = 20)	65 (40.8–84.6)	97.8 (95.6–99.1)	15 (3.2–37.9)	96.5 (94.4–97.9)

CIS, carcinoma in situ; NPV, negative predictive value. \*Three patients had CIS alone, seven patients had co-occurring papillary lesions.

**Fig. 2** Comparison of sensitivity (95% CI) of ADXBLADDER vs cytology by recurrent tumour classification: in all tumours (\**P* < 0.001), low-grade tumours (\*\**P* = 0.02), high-grade tumours (\*\*\*)*P* = 0.04) and Excl pTa low-grade (\*\*\*\**P* = 0.006).



A. Kennedy are employees of Arquer Diagnostics holding share options in the company. The funder of the study assisted the authors in study design, data collection, data analysis, data interpretation, writing of the report, and the decision to submit the paper for publication. P. Gontero, M. Roupret, E. Montanari, F. Longo, T. Dudderidge, J. Palou and S. R. C. McCracken, had access to the raw data. The corresponding author had full access to all the data and had the final responsibility to submit for publication.

**References**

- 1 Papanicolaou GN, Marshall VF. Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. *Science* 1945; 101: 519–20
- 2 Sathianathen NJ, Butaney M, Weight CJ, Kumar R, Konety BR. Urinary biomarkers in the evaluation of primary hematuria: a systematic review and meta-analysis. *Bladder Cancer* 2018; 4: 353–63
- 3 Lotan Y, Roehrborn CG. Sensitivity and specificity of commonly available bladder tumor markers versus cytology: results of a comprehensive literature review and meta-analyses. *Urology* 2003; 61: 109–18

- 4 Freifeld Y, Lotan Y. Effect of blue-light cystoscopy on contemporary performance of urine cytology. *BJU Int* 2019; 124: 251–7
- 5 Lotan Y, O’Sullivan P, Raman JD *et al.* Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. *Urol Oncol* 2017; 35: 531.e15–22
- 6 Barkan GA, Wojcik EM, Nayar R *et al.* The Paris system for reporting urinary cytology: the quest to develop a standardized terminology. *Adv Anat Pathol* 2016; 23: 193–201
- 7 Soria F, Droller MJ, Lotan Y *et al.* An up-to-date catalog of available urinary biomarkers for the surveillance of non-muscle invasive bladder cancer. *World J Urol* 2018; 36: 1981–95
- 8 Stoeber K, Halsall I, Freeman A *et al.* Immunoassay for urothelial cancers that detects DNA replication protein Mcm5 in urine. *Lancet* 1999; 354: 1524–5
- 9 Stoeber K. Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. *CancerSpectrum Knowl Environ* 2002; 94: 1071–9
- 10 Kelly JD, Dudderidge TJ, Wollenschlaeger A *et al.* Bladder cancer diagnosis and identification of clinically significant disease by combined urinary detection of Mcm5 and nuclear matrix protein 22. *PLoS One* 2012; 7: e40305
- 11 Dudderidge T, Stockley J, Nabi G *et al.* A novel, non-invasive test enabling bladder cancer detection in urine sediment of patients presenting with haematuria—a prospective multicentre performance evaluation of ADXBLADDER. *Eur Urol Oncol* 2020; 3: 42–6.
- 12 Daneshmand S, Bazargani ST, Bivalacqua TJ *et al.* Blue light cystoscopy for the diagnosis of bladder cancer: results from the US prospective multicenter registry. *Urol Oncol Semin Orig Investig* 2018; 36: 361.e1–6
- 13 Pichler R, Tulchiner G, Fritz J, Schaefer G, Horninger W, Heidegger I. Urinary UBC rapid and NMP22 test for bladder cancer surveillance in comparison to urinary cytology: Results from a prospective single-center study. *Int J Med Sci* 2017; 14: 811–9
- 14 Yoder BJ, Skacel M, Hedgepeth R *et al.* Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: a prospective study with focus on the natural history of anticipatory positive findings. *Am J Clin Pathol* 2007; 127: 295–301
- 15 Palou J, Brausi M, Catto JWF. Management of patients with normal cystoscopy but positive cytology or urine markers. *Eur Urol Oncol* 2019; 1–7. <https://doi.org/10.1016/j.euo.2019.06.017>

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Abbreviations: AUC, area under the receiver-operating characteristic curve; BCa, bladder cancer; CIS, carcinoma in situ; NMIBC, non-muscle-invasive bladder cancer; NPV, negative predictive value; TURBT, transurethral resection of bladder tumours.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Receiver-operating characteristic (ROC) curve (A) all tumours; (B) non pTaLG.

**Fig. S2.** Comparison of the detection of NMIBC recurrences by ADXBLADDER vs cytology in all tumours (A), low-grade tumours (B), CIS (C) and high-grade tumours (D).