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DOI:

[10.1016/j.euo.2019.06.006](https://doi.org/10.1016/j.euo.2019.06.006)

Publication date:

2019

Document Version

Version created as part of publication process; publisher's layout; not normally made publicly available

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Dudderidge, T., Stockley, J., Nabi, G., Mom, J., Umez-Eronini, N., Hrouda, D., ... McCracken, S. R. C. (2019). A Novel, non-invasive Test Enabling Bladder Cancer Detection in Urine Sediment of Patients Presenting with Haematuria: A Prospective Multicentre Performance Evaluation of ADXBLADDER. *European Urology Oncology*. <https://doi.org/10.1016/j.euo.2019.06.006>

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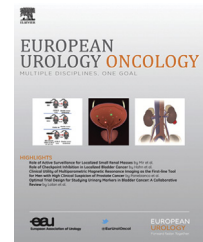
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European Association of Urology



A Novel, non-invasive Test Enabling Bladder Cancer Detection in Urine Sediment of Patients Presenting with Haematuria—A Prospective Multicentre Performance Evaluation of ADXBLADDER

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Article info

Article history:

Accepted June 7, 2019

Associate Editor:

Ashish Kamat

Keywords:

Bladder cancer
Diagnosis
Urine test
Enzyme-linked immunosorbent assay

Abstract

Bladder cancer is the sixth most commonly diagnosed cancer in the European Union. Here, we evaluate the performance of a novel, commercially available enzyme-linked immunosorbent assay utilising MCM5 antibodies (ADXBLADDER; Arquer Diagnostics Ltd, Sunderland, UK) for the detection of bladder cancer, in a blinded, prospective study of 856 patients, across seven centres, presenting with haematuria. The results were compared with the patients' clinical data and final diagnosis as defined by the results of the imaging and cystoscopy, with a prevalence of bladder cancer of 8.6%. ADXBLADDER detected bladder tumours in 54/74 cancers, giving overall sensitivity of 73.0% and an overall negative predictive value (NPV) of 96.4%. Sensitivity and NPV of ADXBLADDER were highest in muscle-invasive bladder cancer, both at 100%, and on analysis of non-pTa (pT1 and above) tumours, the sensitivity for detection was 97% with an NPV of 99.8%. A subset of 173 patients had matching cytology data; of these patients, 18 were positive for bladder cancer. ADXBLADDER detected 16/18 of these cancers, whilst cytology was positive in only four of 18, providing evidence that ADXBLADDER may be a more sensitive test for bladder cancer than standard urine cytology.

Patient summary: We conducted a large clinical study of a novel, simple urine test (ADXBLADDER), which measures a protein (MCM5) in urine and can be used to detect bladder cancer in patients. We recruited 856 patients and demonstrated that the new urine test can detect bladder cancer with a high degree of accuracy, performing better than the most commonly used urine test—urine cytology. In conclusion, this novel ADXBLADDER urine test can be used to help detect bladder cancers and it can replace the current, standard urine test.

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<https://doi.org/10.1016/j.euo.2019.06.006>

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Please cite this article in press as: Dudderidge T, 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* (2019), <https://doi.org/10.1016/j.euo.2019.06.006>

Bladder cancer is the sixth most commonly diagnosed cancer in the European Union (EU), with up to 70% of bladder cancers recurring within the 1st year [1]. The prevalence of this condition, and the nature of its management and follow-up make it one of the most expensive cancers, costing the EU €4.9 billion in 2012 and representing 3% of all cancer costs in the EU [2]. Patients with a primary, single, small TaG1 tumour have a very low chance of progression (<1%), whilst the risk of progression is much higher in patients with stage T1 disease, ranging from 8% to 34%, depending on the depth of invasion of the lamina propria [3,4]. Urine cytology is often used as an aid to cystoscopy in the diagnosis of bladder cancer; however, given the low sensitivity of cytology for low-grade tumours (16%) [3], a negative cytology result is not indicative of the absence of a tumour. Additionally, cytological interpretation is user dependent, with evaluation hampered by low cellular yields, urinary tract infections, and stones, underlining the current importance of the use of diagnostic imaging in conjunction with cystoscopy [5].

MCM5 is an established biomarker in bladder cancer, with detection of MCM5 in the urine of patients with bladder cancer demonstrating excellent sensitivity as well as a very high negative predictive value (NPV) [6,7]; however, earlier research methods were complicated and not suitable for clinical use. Here, we evaluate for the first time the performance of a novel, commercially available enzyme-linked immunosorbent assay (ELISA) utilising MCM5 antibodies, (ADXBLADDER; Arquer Diagnostics Ltd, Sunderland, UK) for the detection of bladder cancer, in a blinded, prospective study of patients presenting with haematuria. MCM5 is a protein marker of replicating cells or cells that have the capability to replicate. Fully differentiated cells lining the bladder, in contact with the urine, do not contain MCM5, so MCM5 is not detectable in urine from a healthy bladder. However, bladder cancer cells are capable of undergoing cell division and therefore contain MCM5. When a tumour is present within the bladder, these cells are shed into the urine stream, resulting in a positive MCM5 ELISA test [6].

Between September 2016 and November 2017, eligible patients presenting with visible or nonvisible haematuria were recruited at urology clinics across seven UK study centres. The patients had no previous diagnosis of bladder, prostate, or renal cancer. Ethical approval was received from the Office for Research Ethics Committee Northern Ireland (15/NI/0258), and informed consent was obtained from all patients prior to the collection of urine samples. A sample size estimate calculation was carried out to determine the performance of ADXBLADDER, based upon the data obtained from feasibility studies and previously published studies [6]. Based upon 80% power, with a 95% confidence level, and assuming a prevalence of 7–10%, a total estimated sample size of 731–1043 patients was required. Patients with known calculi or active prostatitis were excluded from the study (previous studies have shown that these conditions may result in false positive MCM5 results due to damage to the epithelium exposing normal expressing MCM5 cells to the urine [6]). Patients with acute or chronic

urinary tract infection and/or active haematuria (visible and nonvisible) were eligible and therefore included in the study. All patients underwent upper urinary tract imaging (either ultrasound or computed tomography) and flexible cystoscopy. A single, full-void urine sample was collected from patients prior to cystoscopy and processed within 4 h of collection. All urine samples >10 ml were included. Urine sediment was collected by centrifugation and was resuspended in ADXBLADDER lysis buffer. Lysates were incubated for 30 min at room temperature to allow complete lysis before being stored at -20°C or below, prior to testing with ADXBLADDER as per the manufacturer's instructions. The results of the ADXBLADDER test were then compared with the clinical data and the final diagnosis as defined by the results of the imaging and cystoscopy.

The demographics of enrolled patients are summarised in [Supplementary Table 1](#). In total, 856 eligible patients were enrolled with a median age of 64 yr (interquartile [IQ] range 54–73 yr), of whom 74 were found to have a bladder tumour (8.6%). Of the patients with bladder tumours, the majority were males (74.3%) with a median age of 75 yr (IQ range 66–80 yr).

ADXBLADDER demonstrated very high sensitivity of 97.0% (95% confidence interval [CI] 84.0–100%) and an NPV of 99.8% (95% CI 98.7–100%) for the detection of bladder tumours of stage pT1 and above. For muscle-invasive bladder cancer, both sensitivity and NPV were 100% (16/16 tumours). In total, ADXBLADDER detected 54/74 cancers, giving overall sensitivity of 73.0% (95% CI 61.40–82.60%), an overall NPV of 96.4% (95% CI 94.50–97.80%), and an overall specificity of 68.4% (95% CI 65.00–71.70%), with an area under the receiver operator characteristic (ROC) curve of 0.75 (95% CI 0.69–0.81; [Supplementary Fig. 2A](#)). Of the 20/74 tumours undetected by ADXBLADDER, 19/20 were pTa tumours and 14/20 were found to have low-grade disease. Reassuringly, in high-grade non-muscle-invasive disease, sensitivity was high at 86% (95% CI 72–95%) with an NPV of 99.8% (95% CI 97.6–99.6%; [Fig. 1A–C](#)).

A subset of 173 patients had available cytology data; of these patients, 18 were positive for bladder cancer. ADXBLADDER detected 16/18 of these cancers, whilst cytology was positive in only four of these 18 cancers, with six of these 18 having atypical/equivocal results, suggesting that ADXBLADDER may be a more sensitive test for bladder cancer than standard urine cytology ([Fig. 1D](#) and [Supplementary Fig. 2B](#)). Patients recruited to the study included those with both visible and nonvisible haematuria. The performance of ADXBLADDER was consistent between both types of haematuria ([Fig. 2B](#)) and with the overall population, with no significant difference between the areas under the ROC curves ([Fig. 2C](#)). This suggests a role for ADXBLADDER in both visible and nonvisible haematuria. Similarly, there were no significant differences in the performance of ADXBLADDER, as assessed by the area under the ROC curve, due to age (<75 vs ≥ 75 yr) or gender ([Fig. 2C](#)).

An ideal diagnostic test for bladder cancer should be easy to perform, with results promptly available to the clinic, providing additional information to better manage the

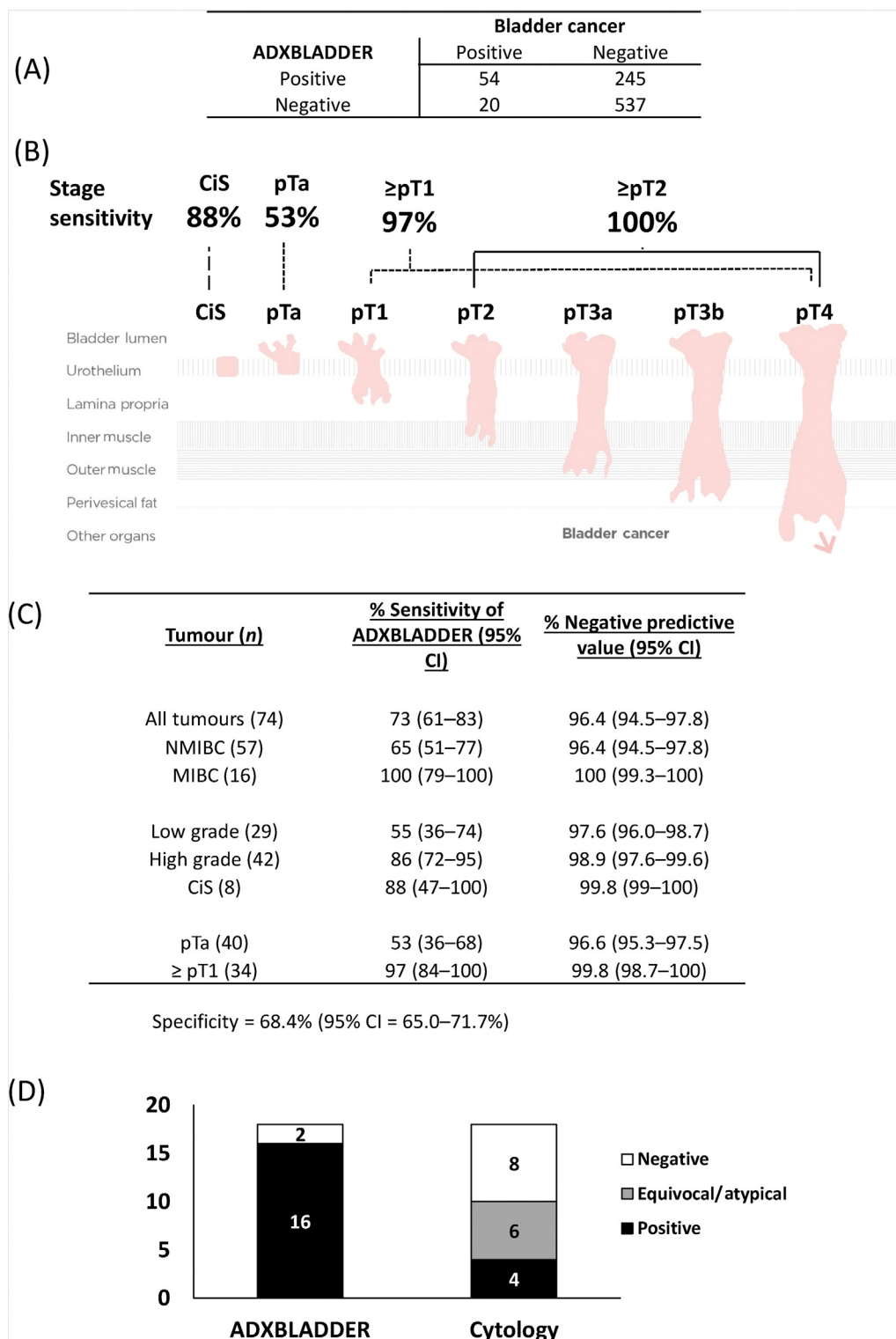


Fig. 1 – (A) A 2 × 2 table of ADXBLEADER performance. (B) Sensitivity of ADXBLEADER associated with tumour stage. (C) Sensitivity and predictive values of ADXBLEADER in patients by grade and stage. (D) ADXBLEADER comparisons with cytology. CI = confidence interval; MIBC = muscle-invasive bladder cancer; NMIBC = non-muscle-invasive bladder cancer.

disease, as well as being cost effective. Furthermore, such a non-invasive test should not be influenced by benign conditions. In the past, urine-based biomarker tests for bladder cancer have not gained widespread acceptance due to their failure to meet or effectively demonstrate such

criteria [8]. The results presented here demonstrate that the performance of ADXBLEADER is not affected by benign conditions, such as urinary tract infections or the presence of haematuria. It has shown a high level of diagnostic sensitivity, in particular for stage pT1 and above (sensitivity

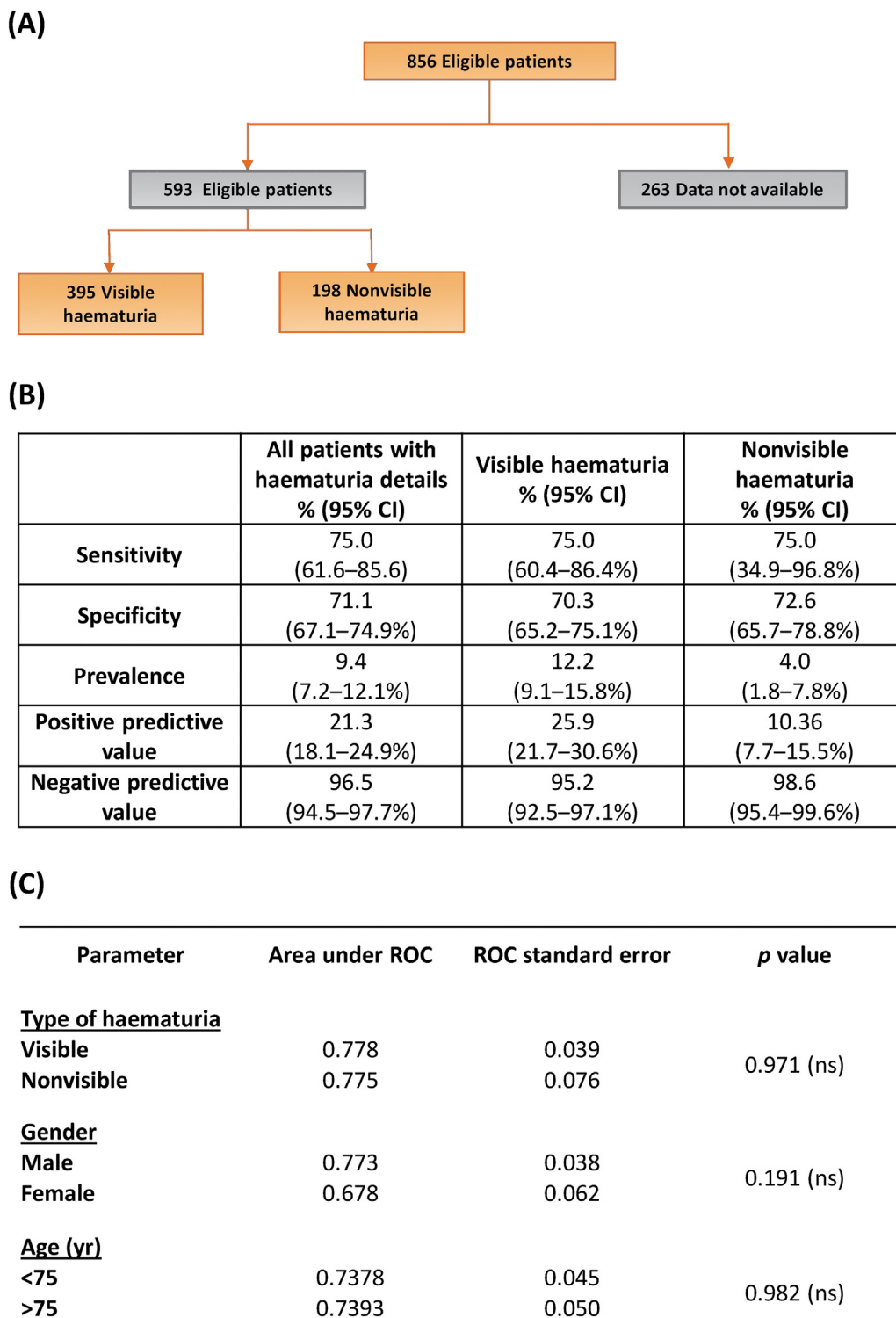


Fig. 2 – (A) Workflow of 593 eligible patients with a documented record of whether haematuria was visible or nonvisible. (B) Performance comparison of ADXBLADDER between patients presenting with either visible or nonvisible haematuria. (C) Comparison of ROC curves of age, gender, and type of haematuria. CI = confidence interval; ROC = receiver operator characteristic.

of 97.0% and an NPV of 99.8%). A favourable comparison to urine cytology demonstrates that ADXBLADDER has the potential to replace cytology as an adjunctive test in bladder cancer diagnosis; however, the authors acknowledge that the number of available cytology comparisons was small

and this study population should be extended to demonstrate a statistically significant increase in the performance of ADXBLADDER when compared with cytology.

The ADXBLADDER non-invasive urine test is an easy-to-perform ELISA test, compatible with general laboratory

equipment available in most hospital laboratories, and can provide results within 3 h, without the need for a pathologist, making it a potential low-cost alternative to cytology. Owing to the prospective nature of this study and the failure to collect cytology data for all patients, a full cost analysis could not be carried out on the potential cost savings. However, further studies, including cost-effectiveness analyses to include cytology as a comparator, are currently underway to define the optimal place where ADXBLADDER could be utilised to strengthen the current diagnostic pathway. Additionally, we postulate that ADXBLADDER also has a role in the surveillance of patients with known, non-muscle-invasive bladder cancer, with a negative test perhaps allowing a greater time period between check cystoscopies. This hypothesis requires a specific study to confirm or deny it; such a study is currently recruiting.

Author contributions: Stuart Robert Crozier McCracken had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Dudderidge, Stockley, McCracken.

Acquisition of data: Dudderidge, Stockley, Nabi, Mom, Umez-Eronini, Hrouda, Cresswell, McCracken.

Analysis and interpretation of data: Dudderidge, Stockley, McCracken.

Drafting of the manuscript: Dudderidge, Stockley, McCracken.

Critical revision of the manuscript for important intellectual content: Dudderidge, Stockley, Nabi, Mom, Umez-Eronini, Hrouda, Cresswell, McCracken.

Statistical analysis: Stockley, McCracken.

Obtaining funding: None.

Administrative, technical, or material support: None.

Supervision: None.

Other: None.

Financial disclosures: Stuart Robert Crozier McCracken certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Dr Jacqui Stockley is an employee of Arquer Diagnostics holding share options in the company.

Mr. T. Dudderidge and Mr. S. McCracken are paid consultants to Arquer Diagnostics. Neither of them has any shareholding in Arquer Diagnostics or any related company.

Funding/Support and role of the sponsor: This study was supported by Arquer Diagnostics Ltd.

Acknowledgements: Clinical performance statistical analysis was performed by Dr. Richard Sylvester, Senior Statistical Scientist Emeritus at European Organisation for Research and Treatment of Cancer (EORTC).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euo.2019.06.006.

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