**A Breakthrough in Non-invasive Bladder Cancer Diagnosis**

**SYMPOSIUM REPORT**

**FROM ARQUER DIAGNOSTICS**

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Prof Juan Palou-Redorta: none known or disclosed.

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Introduction - Prof Juan Palou-Redorta

The 2017 National Comprehensive Cancer Network (NCCN) Guidelines outline the diagnostic procedures to be undertaken in patients with haematuria, including cystoscopy, cytology, abdominal/pelvic CT or MRI before TURBT and imaging of upper tract collecting system. (1)

None of the current diagnostic approaches, however, can be considered ideal. While ultrasound has a sensitivity for detecting bladder cancer of 85%, and a positive predictive value of 98.8% for tumours greater than 1 cm, sensitivity decreases to less than 40% for tumours smaller than 5 mm. (2)

In a study of 435 gross haematuria patients (12.4% of which had tumours), Computed Tomography Urography (CTU) demonstrated a sensitivity of 87%, a specificity of 99% and a negative predictive value (NPV) of 98%. In the same study, flexible cystoscopy was found to have a sensitivity of 87%, specificity of 100% and NPV of 98%. In another study of 150 patients it was shown that CT had a sensitivity and specificity of 61.5% and 94.9% respectively whilst MRI had a sensitivity and specificity of 79.9% and 93.4%. (3)

Given that cytology sensitivity for low grade tumours is very poor (around 25 to 30%), negative cytology results cannot be considered to exclude the presence of tumours, underlining the importance of also undertaking CT scans and cystoscopy. (5)

European Association of Urology (EAU) guidelines state that cystoscopy represents an important step in the diagnosis and management of bladder cancer. However, the guidelines recognize that the quality of cystoscopy is operator dependent, and furthermore that it is an invasive procedure. From the Non Muscle Invasive Bladder Cancer (NMIBC) EAU guidelines it is generally accepted that none of the currently available tests can be considered adequate to replace cystoscopy. (6)

While numerous urine markers have been developed, none have been recommended for primary detection of bladder cancer to date. One study, however, showed that cystoscopy detection rates for urothelial carcinoma were significantly improved when urologists had been made aware of positive urine tests results compared to when they had not (p<0.001). (7)

Adherence to the guidelines recommended care is also an issue. In a study involving 4,545 patients with high-grade non-muscle invasive bladder cancer only one subject received adequate follow-up related to cystoscopy, cytology, and adjuvant treatment. (8). Furthermore, the authors commented that the current surveillance schedule of cystoscopy and cytology every three months and imaging every other year has not been adequately tested (8).

The advantages of using tumour markers for bladder cancer surveillance include the possibility to reduce the frequency of cystoscopy, as well as costs. Disadvantages include the possibility of missed recurrences and false positives which could result in patient anxiety and the cost of additional work-ups. While several markers have been developed the technologies have not proven to be clinically useful due to problems with sensitivity, specificity, and cost.
**MCM5 and the latest ADXBLADDER study – Mr. Stuart McCracken**

Minichromosome Maintenance Protein Complex (MCM) represents a component of the pre-replication complex that forms at the beginning of cell replication/proliferation. It is a eukaryotic DNA helicase complex required specifically for the formation of the DNA replication fork. The MCM complex consists of six related polypeptides (MCM2 to MCM7) that form a hexameric ring structure (6-8).

Over the last 20 years MCM proteins have been studied as markers of cancer. **ADXBLADDER** is a new test for bladder cancer that is built around the detection of the MCM5 protein. While some proliferation markers (such as Ki67) are markers only of actively proliferating cells, MCM5 identifies both cells that are replicating and those retaining the capability to replicate (i.e. those which are not terminally differentiated). However, terminally differentiated cells, such as those on the surface of the bladder epithelium are MCM5 negative.

In healthy people, cells that are MCM5 positive are confined to the stem cell compartment (where they are undifferentiated), however as they undergo differentiation to become the bladder surface epithelial cells, MCM5 expression is lost. Consequently, all cells at the surface, lining the bladder in a healthy individual are MCM5 negative. In cancer, however, there is uncontrolled cell growth with arrested differentiation, resulting in cells throughout the epithelium continuing to be MCM5 positive [See Figure 1].

**Figure 1** [Adapted from Williams GH, Stoeber K. Cell cycle markers in clinical oncology. Current Opinion in Cell Biology. 2007, 19: 672-679.]
Important features of the ADXBLADDER test include providing a yes/no result (unlike cytology which is subjective and can return equivocal results). In addition, results are unaffected by the presence of red blood cells, inflammatory cells or bacteria. The explanation for this is that red blood cells and inflammatory cells are terminally differentiated cells and therefore do not express MCM5, whilst bacteria (responsible for urinary tract infections) do not express MCM5.

There is, however, a potential for false positives in patients with bladder or kidney stones or those who have recently undergone urological manipulations (such as cystoscopy) since such circumstances may disrupt the bladder epithelium and expose undifferentiated cells containing MCM5 that lies beneath the bladder surface.

ADXBLADDER uses a patented sandwich ELISA employing two anti-MCM5 mouse monoclonal antibodies to detect the MCM5 antigen in urine sediment. The test, which can be undertaken in most hospital laboratories, provides a result within three hours, thus meeting the criteria of One-Day Haematuria Clinics.

A blinded prospective diagnostic study of ADXBLADDER was carried out on the entire population attending haematuria clinics, with the primary objective of validating the performance of the urine test in bladder cancer. After the research nurse had explained the study and the patient consented they were asked to provide a urine sample before undergoing routine assessments which for non-visible haematuria included cystoscopy, ultrasound and x-ray and for visible haematuria included cystoscopy and CT urograms. The ELISA results were then evaluated and compared to the standard test results (See Figure 2). For the study patients were asked to provide 50 mL of urine, although for the commercialized test only 10mL is needed.
Study exclusion criteria included patients with known calculi within the urogenital system, those previously diagnosed with bladder, prostate or renal cancer, and those who had urinary tract instrumentation 14 days prior to the test.

Between August 2016 and February 2017, the study was carried out at seven UK sites. Altogether 802 patients were recruited, although 224 were excluded from the analysis, largely due to one site not collecting full void urine. Of the 577 eligible subjects, who had a median age of 63 years, 56% were male and 44% female. Results showed:

- Bladder cancer was diagnosed in 7.96% of tested patients (n=46) of whom 76% (n=35) were men and 24% (n=11) female.
- The overall sensitivity for the ADXBLADDER test was 76% (High risk 92%, muscle invasive group 100%, intermediate risk 75%, and low risk 50%). (See Figure 3)
- The sensitivity for the combined high risk and muscle invasive groups was 95% (high risk groups 92%, muscle invasive groups 100%).
- The overall specificity was 69% and NPV was 97%.

The high NPV of 97% offers reassurance to clinicians since if they tell their patients they have a negative result they can be 97% sure that they do not have bladder cancer.

![Performance Evaluation of ADXBLADDER-Sensitivity by Risk](image)
Only two of the study sites routinely undertook cytology testing, consequently only 10 subjects diagnosed with bladder cancer had accompanying cytology data. Of these, eight (80% sensitivity) were shown to be positive with ADXBLADDER compared to only two (20% sensitivity) with cytology testing.

![Performance Evaluation of ADXBLADDER-Sensitivity of ADXBLADDER vs Cytology](image)

*Only 2 sites collected cytology data as routine so only 10 positives have cytology data*

Addressing performance of ADXBLADDER in other cancers, five patients in the study were diagnosed with upper urinary tract transitional cell carcinoma (TCC) four of whom tested positive for MCM5.

In summary, ADXBLADDER is a simple urine-based test for bladder cancer diagnosis that is non-invasive. Sample collection is easy and requires only 10mL of urine. ADXBLADDER is capable of delivering results in three hours using standard ELISA laboratory equipment available in most hospitals. ADXBLADDER has a NPV greater than 97% and a sensitivity of 95% in high risk non-muscle invasive bladder cancer and muscle invasive bladder cancer.

**Clinical application of ADXBLADDER - Mr. Tim Dudderidge**

The ideal diagnostic test for bladder cancer should be capable of being performed easily and promptly in a clinical environment, provide additional information that helps clinicians to better manage the disease, make the information available in an efficient and timely manner and be cost-effective (10). In the past, molecular marker tests for bladder cancer have not gained widespread acceptance since they failed to demonstrate such criteria effectively.
One of the great strengths of ADXBLADDER is the high NPV, providing patients with the reassurance that if their test is negative there is only a 3% chance of missing cancer. Additionally, unlike many previous tests ADXBLADDER is not affected by UTIs and haematuria, allowing patients with these conditions to use the test. The finding that ADXBLADDER can identify upper tract TCC may additionally avoid the need for unnecessary CT urograms.

One of the reasons none of the previous urine biomarkers succeeded in being adopted was due to tests being studied in isolation and not in combination with imaging modalities. The threshold for acceptance was high as they were required to compete against a combination of tests, such as cystoscopy, CT urograms, cytology and ultrasound. Since current pathways combine different approaches to bladder cancer testing, there is no reason why urinary markers should not be integrated into pathways with other tests.

One possibility is that ADXBLADDER could be used in place of urine cytology since the new test offers advantages including being more accurate, easier to deliver and having no inter-observer errors. ADXBLADDER might also be used as a stand-alone test for patients who do not meet current criteria for cystoscopy and CT scans, such as haematuria that resolves after a urine infection and people under 60 with non-visible haematuria and UTI. While such patients do not meet the current threshold for cystoscopy many would like the reassurance of knowing no cancer is present. The test could also play a valuable role excluding bladder cancer in general screening at well-man and well-woman clinics (particularly for smokers), and also for regular screening of high-risk occupations (such as hairdressers, and rubber and dye industry workers).

Combining the ADXBLADDER test with ultrasound offers the possibility of additive levels of sensitivity and could avoid the need for unnecessary cystoscopy and CT scans. Calculating the potential combined sensitivity of ADXBLADDER (76%) with the sensitivity of bladder ultrasound (80%) could provide a sensitivity of 96.4%, which would rise to 99.1% if a second ADXBLADDER test were undertaken.

Additionally, ADXBLADDER could be used for surveillance of recurrences in patients who have been treated for bladder cancer. A pan-European trial is currently under way to investigate this potential.

**Question and answer session**

Addressing a question of what to do with negative results, Mr. Dudderidge said that clinicians would need to use their clinical judgment according to patient risk. In a 60-year-old smoker with visible haematuria, he said, it would be difficult to take no further action after a negative test. However, he felt certain that the introduction of the test would lead to a decrease in unnecessary cystoscopies.

Mr. McCracken felt that there was already sufficient evidence for ADXBLADDER to replace urine cytology. While the test will have utilities for men and women, he said, the fact that UTIs have no influence on the result represents a ‘real boom’ for women. With non-visible haematuria, he felt, it would be sufficient to combine the test with good quality ultrasound scans of the bladder and kidneys; while with visible haematuria the test could be combined with cystoscopy and ultrasound.
References


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