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Diagnostic accuracy of MCM5 for the detection of recurrence in non muscle invasive bladder cancer follow up: a blinded, prospective cohort, multicentric European study.

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ABSTRACT

Purpose

Detection of MCM5 containing cells in urine has been shown to be indicative for the presence of a bladder tumour in primary diagnosis. The objective of this study was to evaluate diagnostic performance of ADXBLADDER in patients undergoing cystoscopic surveillance in Non Muscle Invasive Bladder Cancer (NMIBC) follow-up.

Materials and Methods

A multicentric prospective blinded study was carried out at 21 European centres in patients undergoing cystoscopy for NMIBC surveillance, diagnosed in the preceding 2 years. Urine was collected from all eligible patients and ADXBLADDER-MCM5 test was performed. Performance characteristics were calculated by comparing MCM5 results to the outcome of cystoscopy plus pathological assessment.

Results

Of 1431 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 non pTaLG tumours (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 non-pTaLG recurrent tumour with a NPV 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 follow-up of NMIBC patients ADXBLADDER is able to exclude the presence of the most aggressive tumours with a NPV of 99%. These results indicate that ADXBLADDER could be incorporated in the follow-up strategy of NMIBC.

INTRODUCTION:

Bladder cancer (BCa) is the 10th 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 patients recurring, making it a major healthcare challenge and

expensive to treat [2]. Currently bladder cancer follow-up relies upon repeat flexible-cystoscopies at regular intervals, the frequency of which is determined by risk categorisation of the patient. Many patients at low risk of 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 of progression are not missed [3]. Whilst 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 tumour. 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-ELISA able to detect MCM5 in urine sediment in BCa patients with haematuria [7]. Our purpose was to evaluate the diagnostic performance of ADXBLADDER in a large, blinded, prospective, multicentric European study of patients undergoing cystoscopic surveillance in BCa follow-up.

MATERIALS & METHODS

Study Design

This was a commercially sponsored, cross-sectional, prospective, blinded study carried out at 21 urology centres in Europe. Overall 1718 patients were enrolled between August 2017 and July 2019, ethical approval was received from all local Research Ethics Committee's (REC Reference: 17/NE/0174) and informed consent obtained from all patients prior to collection of urine samples. All eligible patients were recruited from urology centres across 21 European study centres (Supplementary Table 1). Patients recruited were over 18 years of age, with a previous (pathologically confirmed) diagnosis of non-muscle invasive bladder cancer (NMIBC) 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 10mL were considered eligible. Collected data included: age at the time of cystoscopy, stage, grade and follow-up. 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 was detected on cystoscopy was confirmed to be a recurrent bladder tumour 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 tumour. 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 prior to cystoscopy and processed within 48 hours of collection. 10-50mL of full void urine was centrifuged for 5 minutes at 1,500g 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 -20^{o}C until testing.

MCM5 testing

To determine MCM5 status, (MCM5-positive/MCM5-negative) urine sediment lysates were subjected to ADXBLADDER (Arquer Diagnostics Ltd, Sunderland) testing as per manufacturer's instructions. All clinicians were blinded to results of ADXBLADDER testing, and laboratory staff performing ADXBLADDER were blinded to the results of cystoscopy. Briefly 100ul of lysate was added to ADXBLADDER micro-titre plates and incubated for 60 minutes at room temperature, a wash step was carried out prior to the addition and 30 minutes incubation of 100ul of ADXBLADDER-Conjugate. A second wash step was carried out prior to incubation for 30 minutes with 100ul of TMB, the reaction was stopped by addition of ADXBLADDER-STOP solution. Optical density (OD) was measured at 450nm and 630nm. Any sample with OD greater or equal to the pre-defined cut-off as per the manufacturer's instructions was considered to be MCM5-positive, samples with OD below this were considered to be MCM5-negative.

Statistical Analysis

A sample size calculation was carried out, It was calculated that a total estimated sample size of 752-1504 was required in order 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 5-10%. 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 Area under the Receiver Operator Curve (AUC). All statistical analyses were performed with STATA (version 12.1), statistical significance was indicated if p values were less than 0.05.

RESULTS:

Between August 2017 and July 2019, 1718 patients attending the urology clinic for cystoscopic surveillance in BCa follow-up gave informed consent and provided urine samples. Median patient age was 73 (IQR 66-80), with 1276 (76.3%) males and 442 females (25.7%). 108 (6.3%) were subsequently found to have had a previous diagnosis of MIBC or to be more than 2 years since a positive TURBT/Biopsy and were therefore excluded as per the protocol. Of the remaining 1610 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 prior to processing), 36 (2.2%) were lost in transit by courier, 15 (0.9%) failed to provide 10mL of urine and 12 (0.7%) did not proceed with cystoscopy. In addition, 2 (0.1%) patients were lost to follow-up and 43 (2.7%) were diagnosed with a recurrent BCa but failed to have any pathological examination of suspicious lesions (where lesion was removed by fulguration, or patient entered active surveillance) and were therefore excluded from analysis. (Figure 1)

Of the remaining 1431 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 (IQR 66-80), with 1062 males (74.2%) and 369 females (25.8%). The majority of the patients (51.6%) had previously been diagnosed with a Low Grade pTa tumour and most were between 12-24 months of a positive TURBT (81.8%). For 459 patients (32.1%) the last treatment received was TURBT, whilst 534 (37.3%) patients had received BCG instillations and 423 (29.6%) had received intravesical chemotherapy (403 of which were Mitomycin-C based). All baseline demographic and clinical characteristics of participants are shown in Table 1.

Overall, 57 of the 127 BCa recurrence positive samples tested positive for MCM5 (Overall Sensitivity of 44.9% (95% CI 36.1% to 54%) whilst 927 of the 1304 BCa recurrence negative samples were negative for MCM5, yielding a specificity of 71.1% (95% CI 68.5% to 73.5%) (Table 2) and an area under the ROC curve of 0.57 (95% CI: 0.51-0.62) (Supplementary Figure 1A). Of the 127 cancers, 41 were non-pTaLG tumours, with MCM5 testing positive in 31 of these patients, demonstrating a sensitivity of 75.6% (95% CI 59.7% to 87.6%) with an area under the ROC curve of. 0.71 (95% CI: 0.62-0.80) (Supplementary Figure 1B) Overall NPV of MCM5 testing was 93.0% (95% CI 91.2-94.5) however MCM5 was able to rule out a non-pTaLG recurrent tumour with a NPV of 99.0% (95% CI 98.2-99.5%) (Table 3, Supplementary Figure 2)[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 Figure 3).

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 (Supplemental Figure 4 and 5 respectively).

43 patients were excluded from final analysis as there was no pathological confirmation of suspicious lesions identified on cystoscopy (Figure 1), either because lesions were removed by fulguration, or a watchful waiting approach was adopted. Analysis of these excluded patients reveals that if these patients were to be considered as positive for BCa recurrence, the sensitivity in patients with non-pathologically confirmed tumours is identical to that of pTaLG tumours (Sensitivity 30.2% (95% CI: 17.2-46.1%) for non-pathologically confirmed tumours vs 30.2% (95% CI: 20.8-41.1%) for pTaLG) (Supplementary Table 2). Overall this would have demonstrated 170 patients as positive for recurrent bladder tumours (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).

DISCUSSION

Current diagnostic tools for detection of recurrent BCa tumours in follow-up include cystoscopy and cytology for high-risk NMIBC patients, as recommended by guidelines. Despite urine tests being known to have a positive impact on quality of follow-up cystoscopy [9], to date they have failed to be

utilised 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 follow-up, to date, carried out at 21 high volume centres across Europe.

This blinded prospective study demonstrated that in follow-up of NMIBC patients, ADXBLADDER, an MCM5 test, was able to exclude the presence of the most aggressive tumours (non-pTaLG) with a NPV of 99%. Whilst 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 tumours shed fewer cells into the urine than larger high grade tumours [10] which may explain the difference in sensitivity of the MCM5 test in detecting recurrent tumours when compared to the diagnostic indication, (45% Recurrence BCa vs 73% Primary BCa), with most recurrent tumours being smaller and low grade [11]. Although sensitivity of the MCM5 test for recurrent pTaLG tumours was lower than that observed for primary tumours [7], small, recurrent, low grade tumours 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 tumours [13,14] Therefore, the observed sensitivity for detecting asymptomatic low risk recurrent NMIBC with an MCM5 test, which will be detected at the next follow-up cystoscopy, would provide a safe and cost-effective strategy in the BCa follow-up pathway.

EORTC 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 carry out more regular cystoscopy than 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 tumour can be ruled out, thereby safely reducing the frequency of cystoscopy and decreasing costs. Furthermore, the prospective nature of this large multicentric study ensures that the observed prevalence's closely reflect the situation in most urology clinics, signifying that an MCM5 test such as ADXBLADDER could be effectively incorporated in the follow-up 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 follow-up regimen and the rhythm of surveillance could be adapted, sparing unnecessary cystoscopies for patients at low risk of progression, with a negative ADXBLADDER result having 'ruled out' a high-risk tumour.

Recently there have been many new biomarkers 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 tumours 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 centres 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 was no follow-up data, there could be no indication if false positives of ADXBLADDER were early detection of a sub-clinical recurrence which 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, therefore the presence of upper tract tumours cannot be ruled out. Whilst the study population is very large and confidence intervals are narrow for most of the reported performance characteristics, the non-pTaLG sensitivity is based on 41 recurrences, therefore the CI are slightly wider, although very much in line with previously reported data for MCM5[7,20]. Further multi-centre 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 non-invasive nature, with no constraint for patients. The exceptionally high NPV for high-risk NMIBC recurrences demonstrated here, may in the future 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 follow-up of NMIBC patients a negative ADXBLADDER test result is able to exclude the presence of the most aggressive tumours with an NPV of 99%. These results indicate that ADXBLADDER could be incorporated in the follow-up strategy of NMIBC as a rule-out test.

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FIGURE AND TABLE LEGENDS

Table 1

Patient Demographics at time of recruitment *NB: Includes patients with CiS alone and Cis with co-occurring papillary tumours, Of the 145 cases. only 23 cases had CiS alone (20 Bladder Cancer Negative, 3 Bladder Cancer Positive)*

Table 2

2 x 2 contingency table for (A) All recurrent tumours and (B) non pTaLG recurrent tumours

Table 3

Performance characteristics of ADXBLADDER by tumour type at recurrence **NB 10 of the CiS cases had co-occurring papillary lesions, 4 cases had CiS alone*

Figure 1

Standards for Reporting of Diagnostic Accuracy (STARD) diagram of patient recruitment and enrollment.

Supplementary Table 1

List of recruiting centres

Supplementary Table 2

Sensitivity for cystoscopy identified tumours with no pathological conformation

Supplementary Table 3

2x2 contingency table for all cystoscopy identified tumours (including non-pathologically confirmed)

Supplementary Figure 1

Receiver Operator Curve for (A) All tumours and (B) non-pTaLG tumours

Supplementary Figure 2

2x2 contingency tables and performance of ADXBLADDER by tumour type **NB Risk group was determined according to EAU guidelines which take into account the EORTC risk tables* [8]

Supplementary Figure 3

2x2 Contingency table and performance according to previous tumour diagnosis

Supplementary Figure 4

Receiver Operator Curve for Males vs Females in (A) All tumours and (B) non-pTaLG tumours.

Supplementary Figure 5

Receiver Operator Curve for <73 years of age vs ≥73 years of age in (A) All tumours and (B) non-pTaLG tumours.

Table 1

Characteristic	N (%) or median (interquartile range)		
	Total Population (n=1431)	Bladder cancer Recurrence Positive (n=127)	Bladder cancer Recurrence Negative (n=1304)
Sex			
Male	**1062** *(74.2)*	**94** *(74.0)*	**968** *(74.2)*
Female	**369** *(25.8)*	**33** *(26.0)*	**336** *(25.8)*
Age (y)	**73** *(66-80)*	**73** *(68-80)*	**73** *(66-80)*
Stage and grade of last TURBT/Biopsy, n (%)			
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 Carcinoma in situ (CiS)*	**145** *(10.1)*	**9** *(7.1)*	**136** *(10.4)*
Time between TURBT and ADXBLADDER test n (%)			
< 3 months	**19** *(1.3)*	**3** *(2.4)*	**16** *(1.2)*
3–12 months	**242** *(16.9)*	**34** *(26.8)*	**208** *(16.0)*
> 12 months	**1170** *(81.8)*	**90** *(70.9)*	**1080** *(82.8)*
Last Treatment received , n (%)			
Bacillus Calmette-Guérin (BCG)	**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)*

Table 1: Patient Demographics at time of recruitment.

**NB: Includes patients with CiS alone and Cis with co-occurring papillary tumours, Of the 145 cases, only 23 cases had CiS alone (20 Bladder Cancer Negative, 3 Bladder Cancer Positive)*

Table 2

(A) All tumours

	Bladder Cancer Recurrence	
ADXBLADDER	Positive	Negative
Positive	57	377
Negative	70	927

(B) Non pTaLG tumours

	Bladder Cancer Recurrence	
ADXBLADDER	Positive	Negative
Positive	31	403
Negative	10	987

Table 2: 2 x 2 contingency table

Table 3

Type of tumour (n)	Sensitivity (%) *(95% CI (%))*	NPV (%) *(95% CI (%))*
All tumours (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)*
Number of tumours		
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)*
Size of tumours		
<1cm (79)	**36.7** *(26.1-48.3)*	**94.9** *(94-95.7)*
1-3cm (23)	**65.2** *(42.7-83.6)*	**99.1** *(98.5-99.5)*
> 3cm (1)	*n/a*	*n/a*
pTaLG (86)	**30.2** *(20.8-41.1)*	**94.0** *(92.3-95.4)*
non-pTaLG (41)	**75.6** *(59.7-87.6)*	**99.0** *(98.2-99.5)*

Table 3: Performance characteristics of ADXBLADDER by tumour type

**NB 10 of the CiS cases had co-occurring papillary lesions, 4 cases had CiS alone*

Figure 1

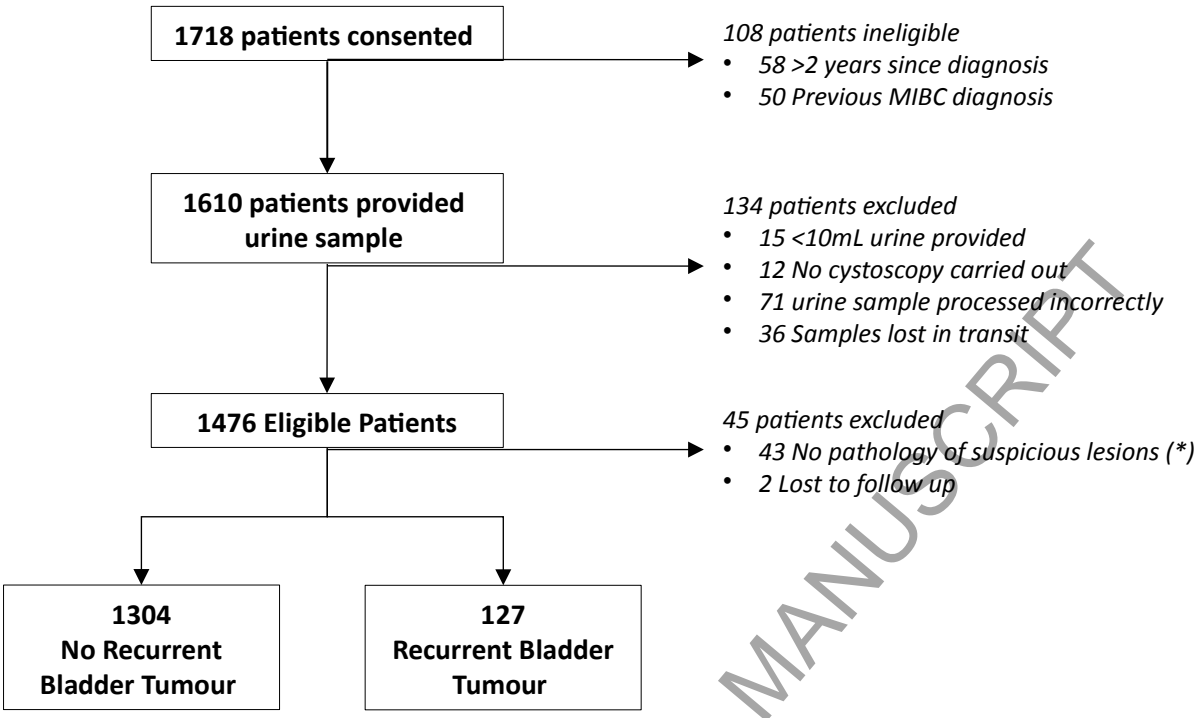

Figure 1 Standards for Reporting of Diagnostic Accuracy (STARD) diagram of patient recruitment and enrollment.