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Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance

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Objectives

To evaluate the diagnostic accuracy of the Xpert Bladder Cancer (BC) Monitor, compared with cystoscopy and cytology in the oncological follow-up of non-muscle-invasive bladder cancer (NMIBC).

Material and Methods

A total of 140 patients with a history of NMIBC undergoing routine surveillance at our institution were enrolled prospectively in this study (ISRCTN study registry number 37210907). Urine cytology was evaluated according to the Paris classification system. In addition, urinary specimens were analysed using the Xpert BC Monitor, which measures five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) using real-time PCR. Descriptive analysis, diagnostic accuracy including sensitivity, specificity, positive (PPV) and negative predictive value (NPV), receiver-operating characteristic curve, and area under the curve (AUC) were calculated.

Results

The overall sensitivity (0.84) and NPV (0.93) of the Xpert BC Monitor were significantly superior to those of bladder washing cytology (0.33 and 0.76; $P < 0.001$). Subgroup analyses confirmed the high sensitivity of the Xpert BC Monitor even in low-grade (0.77) and pTa (0.82) disease compared with barbotage cytology (low-grade: 0.13; pTa: 0.21). The overall specificity of the Xpert BC Monitor and barbotage cytology was similar (0.91 vs 0.94; $P = 0.41$). Combining the Xpert BC Monitor with barbotage cytology (AUC = 0.85) did not enhance diagnostic performance compared with the performance of the Xpert BC Monitor alone (AUC = 0.87).

Conclusion

In this study, we report for the first time that the Xpert BC Monitor, a new mRNA-based urine test, outperforms cytology with regard to sensitivity and NPV, even in low-grade and pTa tumours, with no reduction of specificity.

Keywords

follow-up, mRNA-based markers, recurrence, surveillance, urine markers, #BladderCancer, #blcsm

Introduction

Bladder cancer (BC) is the second most common urological cancer entity, with an estimated occurrence of 430 000 new cancers worldwide in 2012 [1]. In 70–75% of cases, the initial disease is classified as non-muscle-invasive bladder cancer (NMIBC), confined to the mucosa or submucosa [2]. Total endoscopic tumour resection is the standard initial treatment of choice; however, even after complete tumour resection, the probability of tumour recurrence is 15–61% in the first year of diagnosis, and 31–78% at 5 years after initial manifestation of the disease, respectively [3–5]. In addition, 5-year tumour progression is observed in up to 45% of cases, especially in patients with high-risk NMIBC which has the highest potential for tumour progression [3–5]. These follow-up data reflect the fact that NMIBC incorporates a wide and heterogeneous spectrum of tumours with a pleotropic molecular landscape, resulting in different prognoses and behaviours [6]. This highlights the importance of comprehensive, standardized and risk-adapted follow-up protocols for patients with NMIBC, including regular cystoscopies and cytologies, as well as regular upper urinary tract imaging (for high-risk tumours) as suggested by the European Association of Urology (EAU) guidelines [4,6].

In general, cytology has a high sensitivity for high grade tumours, but is limited by its low sensitivity (16%) in low-grade tumours [7,8]. Even when using the Paris classification system, the sensitivity of urinary cytology for detecting low-grade NMIBC was low, ranging from 21% to 53% in an

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inter-observer variability analysis [9]. A variety of commercially available urinary molecular markers have been introduced for detecting BC recurrence; these are thought to reduce or ideally replace follow-up cystoscopies and cytologies on follow-up [10,11]; however, the majority of the tested urinary-based markers (e.g. Urovysion, Nuclear matrix Protein 22 [NMP22], Immunocyt/uCyt) had a higher sensitivity, but a lower specificity than cytology, and were unable to replace cystoscopy in BC surveillance [4,10,11].

The Xpert BC Monitor is an mRNA-based urinary marker test for BC surveillance which measures the levels of five target mRNAs from a voided urine sample by real-time RT-PCR. The Xpert BC Monitor automates and integrates sample processing, nucleic acid amplification, and the detection of target sequences. The test requires <2 min of hands-on sample preparation time and a total turnaround PCR time of ~90 min. Moreover, the disposable PCR cartridges hold all PCR reagents and host the entire PCR process.

The aim of the present prospective study was to evaluate, for the first time, the impact, feasibility and diagnostic accuracy of the Xpert BC Monitor compared with the 'gold standard' of cystoscopy and urine cytology, applied in the oncological follow-up of patients with a history of NMIBC.

Materials and Methods

After approval from the local ethics committee (study number AN2016-0056; 360/4.7 and 368/5.12 [3954a]), the medical records of patients with a history of NMIBC undergoing routine follow-up at our department between January 2017 and July 2017 were reviewed prospectively. The follow-up protocol included cystoscopy (flexible in men, rigid in women) in combination with voided urine and bladder washing cytology as gold standard. The frequency of follow-up cystoscopies and upper urinary tract imaging was based on the current EAU guidelines [4]. Urinary cytology was evaluated according to the Paris classification system [12,13].

In addition to urine cytology, urinary samples were analysed using the Xpert BC Monitor test (Cepheid, Sunnyvale, CA, USA), measuring the levels of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) by RT-PCR according to the manufacturer`s protocol. Briefly, urine samples were supplemented within 1 h of collection with the Xpert Urine Transport Reagent Kit (Cepheid) by transferring 4.5 mL of voided urine to the urine transport reagent tube and inverting three times to mix. Subsequently, 4 mL of pretreated urine was transferred to the disposable cartridge. All reagents needed for sample preparation and RT-PCR were present in the self-contained cartridge. Automated processing included capturing cells on a filter, lysis by sonication, addition of released nucleic acid to dry RT-PCR reagents, transfer to the reaction chamber, multiplex RT-PCR and detection. ABL1 served as a sample adequacy control to ensure that the sample contained human cells and human RNA. The ABL1 signal was required for a valid test result. An ABL1-negative signal indicated that the sample did not contain sufficient human cells or had degraded, yielding invalid results. Before the start of the PCR, the GeneXpert Instrument System was used to measure the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling in the cartridge, probe integrity, and dye stability. A 'Cepheid internal control', designed to detect sample-associated inhibition of the real-time RT-PCR, was included in each cartridge. The qualitative test provided a 'negative' or 'positive' result based on the results of a linear discriminant analysis (LDA) algorithm (total LDA within the valid range of –20 to 20), which uses the cycle threshold results of the five mRNA targets. A positive result is achieved when ABL1 cycle threshold and total LDA are within the valid range, the total LDA (the result of an algorithm that uses the cycle threshold values of ABL1, ANXA10, UPK1B, CRH and IGF2) is equal to or above the cut-off point, the sample passes the probe check control, and Cepheid internal control results are ignored (assay targets in positive samples can interfere with this control). External quality controls (Xpert Bladder Cancer Panel C104, Maine Molecular Quality Controls, Inc., Saco, ME, USA) were performed to monitor the performance of the Xpert BC Monitor, including low positive controls (containing synthetic RNA transcripts carrying target sequences of CRH, IGF2, UPK1B, ANXA10 and ABL1) and negative controls (containing synthetic RNA transcripts carrying target sequence of the endogenous control ABL1 only).

Statistical analyses were performed using SPSS software, version 22 (IBM Corp., Armonk, NY, USA). A significance level of $\alpha = 0.05$ (two-tailed) was applied for all *P* values. To assess the diagnostic accuracy of cytology, Xpert BC Monitor, and combined testing as compared with cystoscopy, data were tabulated in contingency tables. The sensitivity, specificity, and positive (PPVs) and negative predictive values (NPVs) were calculated, with stratification by descriptive variables, and histopathological factors (in case of recurrence). The 95% CIs were calculated according to the DeLong method for area under the curve (AUC) and exact binomial CIs for the other variables. Sensitivity and specificity were compared using McNemar's test. PPV and NPV were compared using the generalized score statistic test by Leisenring et al. [14]. Receiver-operating characteristic (ROC) curves were plotted and the AUC was calculated and tested for significance using the Mann–Whitney *U*-test.

Results

A total of 155 urine samples from 140 patients (mean [range] age 71.0 [31–91] years) with a history of NMIBC were prospectively collected during oncological follow-up of NMIBC and were available for further urine analysis. Invalid

test results attributable to a negative ABL1 signal necessitated a repeat Xpert BC monitor analysis in 15 patients (10.7%). Of the 140 patients, 78 (55.7%) underwent surveillance after primary tumour resection and 62 (44.3%) after recurrent disease. Of the latter, 37 (59.7%) had had a single prior recurrence. On oncological follow-up during the investigation, a new tumour recurrence was detected in 43 (30.7%) of the 140 patients. Descriptive and histopathological patient characteristics of the previous primary tumour (last transurethral resection of the bladder) and the new recurrent tumour are shown in Table 1.

The Xpert BC Monitor test was significantly superior to bladder washing cytology in terms of overall sensitivity (84% vs 33%; $P < 0.001$) and NPV (93% vs 76%; $P < 0.001$), and had a similar high overall specificity (91% vs 94%; $P = 0.41$; Tables 2 and 3). Subgroup analysis of the diagnostic performance of the Xpert BC Monitor, voided urine and bladder washing cytology, as well as combined testing were performed across patient demographics and tumour characteristics (Table 2). While the sensitivity of the Xpert BC Monitor was 100% in patients with high-grade tumours compared with 83% of bladder washing cytology, it was also significantly higher in low-grade (77% vs 13%), Ta (82% vs 21%), single (68% vs 18%) and low-volume (75% vs 29%) tumours (<3 cm) using the Xpert BC Monitor compared with bladder washing cytology (Table 2). Changes in the diagnostic performance of the Xpert BC Monitor, comparator tests, and combined testing are shown in detail in Table 3.

Table 1 Descriptive and histopathological characteristics of patients undergoing surveillance after a history of non-muscle-invasive bladder cancer.

Characteristic	Primary previous tumour (*N* = 140) *n* (%)	Recurrent tumour (*N* = 43) *n* (%)
Age		
≤65 years	36 (25.7)	9 (20.9)
>65 years	104 (74.3)	34 (79.1)
Sex		
Female	34 (24.3)	7 (16.3)
Male	106 (75.7)	36 (83.7)
Number of tumours		
Single	72 (51.4)	22 (53.7)
Multiple	68 (48.6)	19 (46.3)
Tumour size		
<3 cm	112 (80)	28 (68.3)
≥3 cm	22 (20)	13 (31.7)
Stage		
Ta	110 (78.6)	33 (76.7)
Tis	8 (5.7)	2 (4.7)
T1	22 (15.7)	6 (14.0)
>T1	0	2 (4.7)
Grade		
Low	97 (69.3)	31 (72.1)
High	43 (30.7)	12 (27.9)
Number of recurrences		
0	78 (55.7)	27 (62.8)
1	37 (26.4)	11 (25.6)
2	20 (14.3)	4 (9.3)
3	5 (3.6)	1 (2.3)
Previous intravesical instillation therapy		
None	81 (57.9)	33 (76.7)
BCG	33 (23.6)	3 (7.0)
Mitomycin C	26 (18.6)	7 (16.3)

The ROC curve analysis also confirmed the diagnostic superiority of the Xpert BC Monitor (AUC = 0.87, 95% CI 0.80–0.94; $P < 0.001$) over voided urine cytology (AUC = 0.55, 95% CI 0.44–0.65; $P = 0.38$) and barbotage cytology (AUC = 0.63, 95% CI 0.53–0.74, $P = 0.013$). As a next step, we analysed the combination of the Xpert BC Monitor with standard NMIBC follow-up including bladder washing cytology. Our data clearly showed that combining the Xpert BC Monitor with barbotage cytology did not enhance diagnostic accuracy compared with Xpert BC Monitor measurement alone (the AUC for the combination was 0.85 vs 0.87 for the Xpert BC Monitor alone; Figure 1).

Discussion

Cytology is a useful addition to cystoscopy in the follow-up of patients with high-grade NMIBC, but is known to have a low sensitivity for detecting low-grade disease in the follow-up after primary tumour manifestation [7,8]. Moreover, subjective and pathologist-dependent cytological interpretation, the inadequacy of specimens because of their limited volume and cellularity [15], lack of consensus for atypical categorization, and the definition of validated criteria for individual categories may explain the variable diagnostic performance of cytology in different classification systems [16]. To address this problem, a novel standardized cytology reporting system with redefined diagnostic categories was introduced recently by the Paris Work Group [12]. Even with this cytological classification, NPVs and sensitivities for low-grade tumours were found to range between 57% and 67%, and 21% and 53% respectively [9]. Although a number of urinary molecular marker tests have been developed to improve diagnostic accuracy, and have been approved by the US Food and Drug Administration, none of the currently available tests have been accepted or incorporated in treatment guidelines [17–19]. Furthermore, no specific urine biomarker is validated in urothelial cancer of the upper urinary tract to improve peri-operative or postoperative risk stratification concerning risk-based surveillance [20].

In contrast to tested urinary marker tests [10,11], urine cytology still achieves the best specificity (94%) [19], and is an important tool for the detection of primary BC in patients with haematuria and in the surveillance of patients with high-grade NMIBC. Although protein-based urine markers such as bladder tumour-associated antigen (BTA) and NMP22 offer improved sensitivity for detecting low-grade tumours compared with cytology, sensitivity remained equivalent for high-grade tumours, with a lower specificity than cytology [21]. Moreover, haematuria, nephrolithiasis, inflammation

Table 2 Comparison of the diagnostic accuracy of voided urine cytology, bladder washing cytology, the Xpert BC Monitor, and combined testing (overall and stratified by patient demographics and recurrent tumour classification).

Urinary test	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Voided urine cytology								
Overall	4	97	0	39	0.09 (0.03, 0.22)	1.00 (0.94, 1.00)	1.00 (0.28, 1.00)	0.71 (0.63, 0.79)
Gender								
Female	0	27	0	7	0.00 (0.00, 0.53)	1.00 (0.82, 1.00)	NaN (0.00, 1.00)	0.79 (0.62, 0.91)
Male	4	70	0	32	0.11 (0.03, 0.26)	1.00 (0.92, 1.00)	1.00 (0.28, 1.00)	0.69 (0.59, 0.77)
Age								
≤65	0	27	0	9	0.00 (0.00, 0.45)	1.00 (0.82, 1.00)	NaN (0.00, 1.00)	0.75 (0.58, 0.88)
>65	4	70	0	30	0.12 (0.03, 0.27)	1.00 (0.92, 1.00)	1.00 (0.28, 1.00)	0.70 (0.60, 0.79)
Number								
Single	0	–	–	22	0.00 (0.00, 0.22)	–	–	–
Multiple	2	–	–	17	0.11 (0.01, 0.33)	–	–	-
Size								
<3 cm	2	–	–	26	0.07 (0.01, 0.24)	–	–	–
≥3 cm	0	–	–	13	0.00 (0.00, 0.34)	–	–	–
Grade								
Low–grade	1	–	–	30	0.03 (0.00, 0.17)	–	–	–
High–grade	3	–	–	9	0.25 (0.05, 0.57)	–	–	–
Stage								
Ta	0	–	–	33	0.00 (0.00, 0.15)	–	–	–
Tis	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
T1	2	–	–	4	0.33 (0.04, 0.78)	–	–	–
>T1	0	–	–	2	0.00 (0.00, 0.91)	–	–	–
Previous intravesical instillation therapy								
Yes	2	49	0	8	0.20 (0.03, 0.56)	1.00 (0.89, 1.00)	1.00 (0.09, 1.00)	0.86 (0.74, 0.94)
No	2	48	0	31	0.06 (0.01, 0.20)	1.00 (0.89, 1.00)	1.00 (0.09, 1.00)	0.61 (0.49, 0.72)
Bladder washing cytology								
Overall	14	91	6	29	0.33 (0.19, 0.49)	0.94 (0.87, 0.98)	0.70 (0.46, 0.88)	0.76 (0.67, 0.83)
Gender								
Female	2	27	0	5	0.29 (0.04, 0.71)	1.00 (0.82, 1.00)	1.00 (0.09, 1.00)	0.84 (0.67, 0.95)
Male	12	64	6	24	0.33 (0.19, 0.51)	0.91 (0.82, 0.97)	0.67 (0.41, 0.87)	0.73 (0.62, 0.82)
Age								
≤65	1	25	2	8	0.11 (0.00, 0.48)	0.93 (0.76, 0.99)	0.33 (0.01, 0.91)	0.76 (0.58, 0.89)
>65	13	66	4	21	0.38 (0.22, 0.56)	0.94 (0.86, 0.98)	0.76 (0.50, 0.93)	0.76 (0.65, 0.84)
Number								
Single	4	–	–	18	0.18 (0.05, 0.40)	–	–	–
Multiple	8	–	–	11	0.42 (0.20, 0.67)	–	–	–
Size								
<3 cm	8	–	–	20	0.29 (0.13, 0.49)	–	–	–
≥3 cm	4	–	–	9	0.31 (0.09, 0.61)	–	–	–
Grade								
Low–grade	4	–	–	27	0.13 (0.04, 0.30)	–	–	–
High–grade	10	–	–	2	0.83 (0.52, 0.98)	–	–	–
Stage								
Ta	7	–	–	26	0.21 (0.09, 0.39)	–	–	–
Tis	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
T1	3	–	–	3	0.50 (0.12, 0.88)	–	–	–
>T1	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
Previous intravesical instillation therapy								
Yes	6	47	2	4	0.60 (0.26, 0.88)	0.96 (0.86, 1.00)	0.75 (0.35, 0.97)	0.92 (0.81, 0.98)
No	8	44	4	25	0.24 (0.11, 0.42)	0.92 (0.80, 0.98)	0.67 (0.35, 0.90)	0.64 (0.51, 0.75)
Xpert BC Monitor								
Overall	36	88	9	7	0.84 (0.69, 0.93)	0.91 (0.83, 0.96)	0.80 (0.65, 0.90)	0.93 (0.85, 0.97)
Gender								
Female	6	24	3	1	0.86 (0.42, 1.00)	0.89 (0.71, 0.98)	0.67 (0.30, 0.93)	0.96 (0.80, 1.00)
Male	30	64	6	6	0.83 (0.67, 0.94)	0.91 (0.82, 0.97)	0.83 (0.67, 0.94)	0.91 (0.82, 0.97)
Age								
≤65	7	27	0	2	0.78 (0.40, 0.97)	1.00 (0.82, 1.00)	1.00 (0.47, 1.00)	0.93 (0.77, 0.99)
>65	29	61	9	5	0.85 (0.69, 0.95)	0.87 (0.77, 0.94)	0.76 (0.60, 0.89)	0.92 (0.83, 0.97)
Number								
Single	15	–	–	7	0.68 (0.45, 0.86)	–	–	–
Multiple	19	–	–	0	1.00 (0.75, 1.00)	–	–	–
Size								
<3 cm	21	–	–	7	0.75 (0.55, 0.89)	–	–	–
≥3 cm	13	–	–	0	1.00 (0.66, 1.00)	–	–	–

Table 2 (continued)

Urinary test	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Grade								
Low–grade	24	–	–	7	0.77 (0.59, 0.90)	–	–	–
High–grade	12	–	–	0	1.00 (0.64, 1.00)	–	–	–
Stage								
Ta	27	–	–	6	0.82 (0.65, 0.93)	–	–	–
Tis	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
T1	5	–	–	1	0.83 (0.36, 1.00)	–	–	–
>T1	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
Previous intravesical instillation therapy								
Yes	10	45	4	0	1.00 (0.59, 1.00)	0.92 (0.80, 0.98)	0.71 (0.42, 0.92)	1.00 (0.88, 1.00)
No	26	43	5	7	0.79 (0.61, 0.91)	0.90 (0.77, 0.97)	0.84 (0.66, 0.95)	0.86 (0.73, 0.94)
Combined Xpert/washing*								
Overall	36	83	14	7	0.84 (0.69, 0.93)	0.86 (0.77, 0.92)	0.72 (0.58, 0.84)	0.92 (0.85, 0.97)
Gender								
Female	6	24	3	1	0.86 (0.42, 1.00)	0.89 (0.71, 0.98)	0.67 (0.30, 0.93)	0.96 (0.80, 1.00)
Male	30	59	11	6	0.83 (0.67, 0.94)	0.84 (0.74, 0.92)	0.73 (0.57, 0.86)	0.91 (0.81, 0.97)
Age								
≤65	7	25	2	2	0.78 (0.40, 0.97)	0.93 (0.76, 0.99)	0.78 (0.40, 0.97)	0.93 (0.76, 0.99)
>65	29	58	12	5	0.85 (0.69, 0.95)	0.83 (0.72, 0.91)	0.71 (0.54, 0.84)	0.92 (0.82, 0.97)
Number								
Single	15	–	–	7	0.68 (0.45, 0.86)	–	–	–
Multiple	19	–	–	0	1.00 (0.75, 1.00)	–	–	–
Size								
<3 cm	21	–	–	7	0.75 (0.55, 0.89)	–	–	–
≥3 cm	13	–	–	0	1.00 (0.66, 1.00)	–	–	–
Grade								
Low–grade	24	–	–	7	0.77 (0.59, 0.90)	–	–	–
High–grade	12	–	–	0	1.00 (0.64, 1.00)	–	–	–
Stage								
Ta	27	–	–	6	0.82 (0.65, 0.93)	–	–	–
Tis	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
T1	5	–	–	1	0.83 (0.36, 1.00)	–	–	–
>T1	2	–	–	0	1.00 (0.09, 1.00)	–	–	–
Previous intravesical instillation therapy								
Yes	10	44	5	0	1.00 (0.59, 1.00)	0.90 (0.78, 0.97)	0.67 (0.38, 0.88)	1.00 (0.88, 1.00)
No	26	39	9	7	0.79 (0.61, 0.91)	0.81 (0.67, 0.91)	0.74 (0.57, 0.88)	0.85 (0.71, 0.94)

Table 3 Changes in sensitivity, specificity, PPV and NPV between the Xpert Bladder Cancer (BC) Monitor alone, voided urine cytology alone, bladder washing cytology alone, and combined (Xpert BC Monitor and bladder washing cytology) testing.

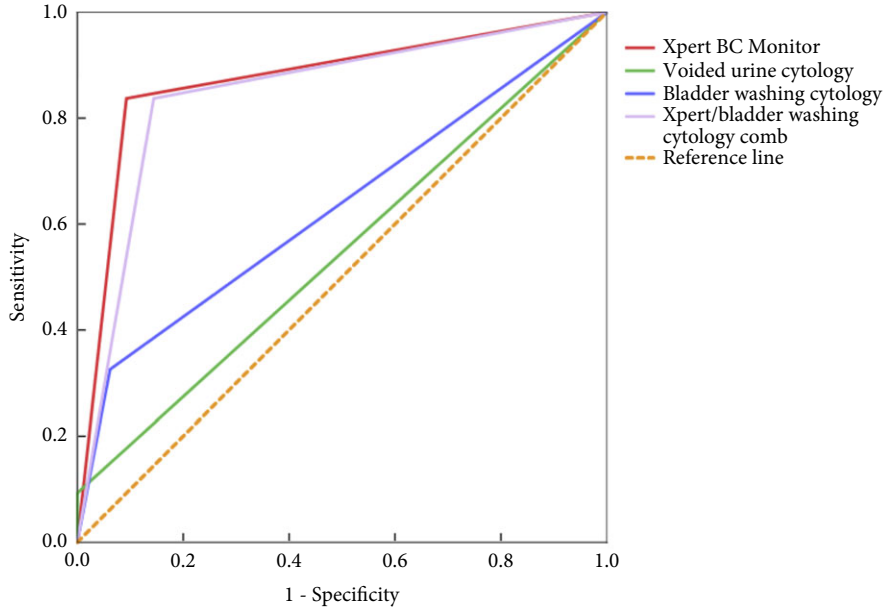
Diagnostic performances	Sensitivity	Specificity	PPV	NPV
Xpert BC Monitor vs voided urine cytology	<0.001***	0.003**	0.05	<0.001***
Xpert BC Monitor vs bladder washing cytology	<0.001***	0.41	0.35	<0.001***
Xpert BC Monitor vs combined Xpert/washing cytology	1.00	0.025*	0.02*	0.078
Voided urine cytology vs bladder washing cytology	0.002**	0.014*	0.034*	0.014*

*PPV, positive predictive value; NPV, negative predictive value. P values are presented according to McNemar's test for sensitivity and specificity, and the generalized score statistic test by Leisenring et al. [14] for PPV and NPV. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.*

and intravesical instillation therapies may cause false-positive results [22,23]. By contrast, the Xpert BC Monitor succeeded in discriminating between tumour stages, tumour grades, tumour size and number of tumours, with no higher rate of false positivity in patients with previous intravesical instillations in our series.

In addition to protein-based urine markers such as BTA, NMP22 and proteins detected on fixed urothelial cells (ImmunoCyt), and chromosomal aberrations detected by fluorescence *in situ* hybridization (UroVysion) [24], a number of urinary RNA-based biomarker panels are being developed and validated at the present time. This is a promising new approach for improving diagnostic accuracy in BC surveillance [24]. The multigene Cxbladder Monitor test measures five mRNA targets (MDK, HOXA13, CDC2, IGFBP5 and CXCR2) by quantitative RT-PCR. It achieved an impressive sensitivity and NPV of 91% and 96% compared with cytology (22% and 87%) for detecting BC in a study comprising 803 patients undergoing surveillance [25]. The test also had a high sensitivity of 86% in Ta low-grade disease

Fig. 1 Receiver-operating characteristic curves and areas under the curve (AUCs), including 95% CIs, calculated for voided urine cytology, bladder washing cytology, the Xpert Bladder Cancer (BC) Monitor and the combination of the Xpert BC Monitor with bladder washing cytology. *P* values obtained using the Mann–Whitney *U*-test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.



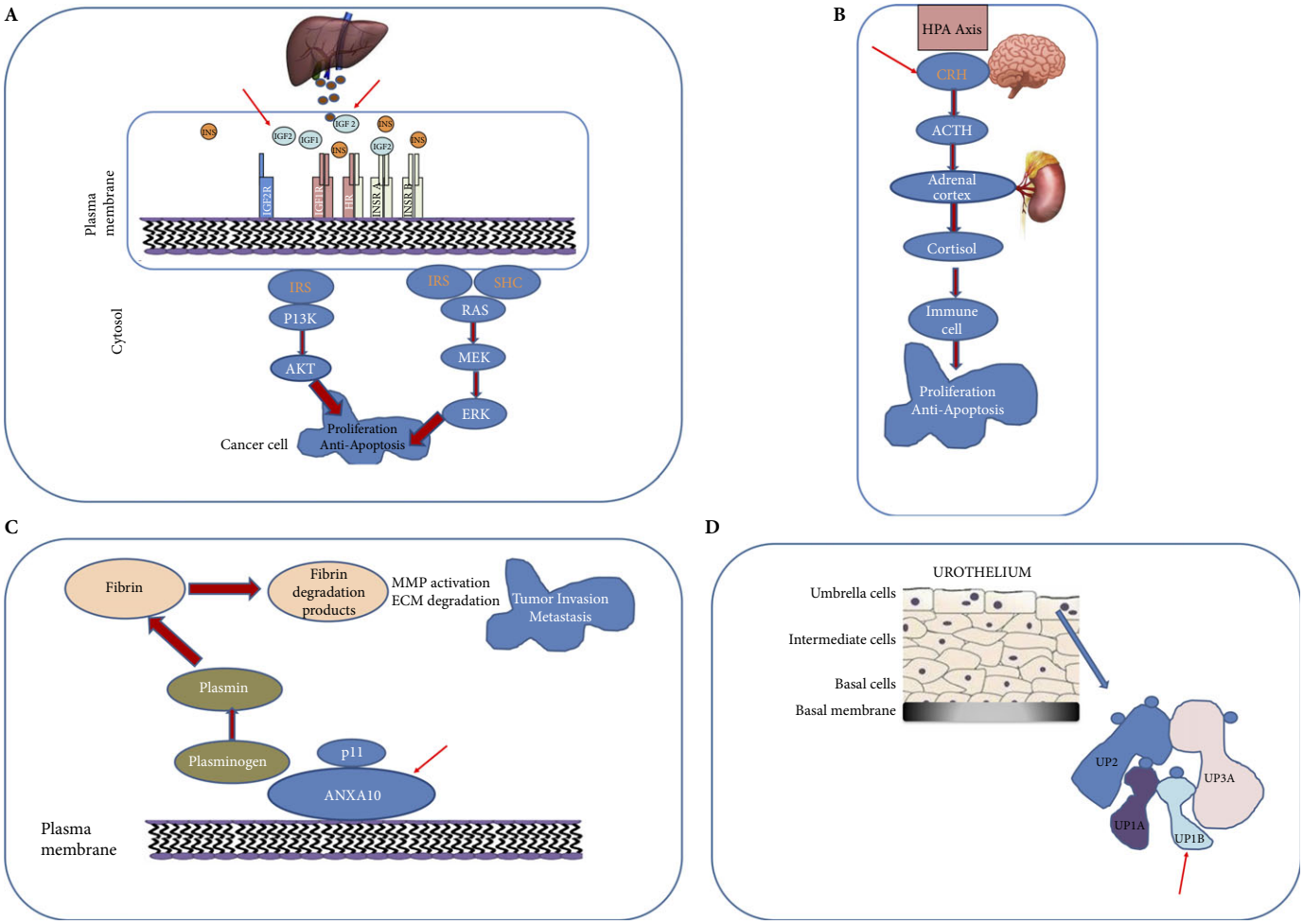
Diagnostic test method	AUC (95% CI)	*P*
Xpert BC Monitor	0.872 (0.800-0.945)	<0.001***
Voided urine cytology	0.547 (0.440-0.653)	0.381
Bladder washing cytology	0.632 (0.525-0.739)	0.013*
Xpert/bladder washing cytology combined	0.846 (0.771-0.922)	<0.001***

[26]. The application of the Cxbladder Monitor in patients with microhaematuria reduced the number of invasive diagnostic procedures such as cystoscopy and CT scans [27]. A novel miRNA signature panel (miR16, miR200c, miR205, miR21, miR221 and miR34a) developed to monitor patients with BC in the active surveillance regime achieved a high overall sensitivity of 88%, but a low specificity of 48%, and also had a limited ability to detect patients with low-volume tumours (AUC = 0.69) [28]. Several years ago, Mengual et al. [29] reported a new urinary gene expression profile including a 12 + 2 gene set panel (ANXA10, AHNAK2, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, and TERT + ASAM and MCM10) that achieved high sensitivity rates of 80% and 93% for the detection of low-grade and high-grade NMIBC, respectively.

Three genes from the Mengual panel [30] (ANXA10, CRH and IGF2) are included in the Xpert BC Monitor test, an expression panel of five genes including ABL1 and UPK1B in addition. Performing literature review, 100 selected tumour markers being differentially expressed in BC were tested by quantitative RT-PCR to identify markers that consistently show upregulation in urine specimens from subjects with BC compared with urine specimens from control subjects. The 10 best biomarkers (UPK1B, IGF2, CRH, ANXA10, ABL1, KRT20, AR, PIK3CA, UPK2 and MGEA5) were evaluated within a training set of 444 urine specimens. Linear modelling was used to reduce the number of markers to the five best-performing tumour markers. The results of expression of the five tumour markers (UPK1B, IGF2, CRH, ANXA10, ABL1) are combined in a linear model algorithm to give a positive or negative result using the Xpert BC Monitor.

The markers used in the Xpert BC Monitor test are related to proliferation and survival (IGF2), neuroendocrine stress response, immunity and inflammation (CRH), cell growth and signal transduction (ANXA10), and epigenetic dysregulation in BC (UPK1B; Figure 2A–D). IGF-2 is a growth factor of the IGF axis which has been linked to the development and progression of various tumour entities [30]. The corticotropin-releasing hormone (CRH) system was initially identified as a hypothalamus-directed mediator of neuroendocrine stress response, while recent studies suggest a link between CRH and the development of solid cancers [31]. Preclinical studies showed the proinflammatory and procarcinogenic nature of CRH family peptides and their receptors, and the fact that they modulate immunity, inflammation and tumour cell growth [32].

Fig. 2 Schematic overview of the functions and pathways of the different markers used in the Xpert Bladder Cancer (BC) Monitor test (CRH, IGF2, UPK1B and ANXA10). mRNAs of these genes are detected by the Xpert BC Monitor. (A) IGF-2 is as a member of the insulin like growth factor family, being involved in cell proliferation and differentiation. Overexpression of IGF-2 resulting from loss of its imprinting results in abnormal signal transduction and/or promoter activation, which are frequently observed in a variety of human tumours such as BC. (B) CRH family peptides and their receptors may modulate inflammation and tumour cell growth. They are proinflammatory and procarcinogenic in inflammation-associated cancers. (C) ANXA10 is a member of the ANX family, being involved in various physiological functions in membrane transport, calcium signalling, cell proliferation and differentiation, with aberrant regulation in neoplasia, supporting tumour development and progression. Depending on the cancer entity, ANX members may act as oncogenes or tumour suppressor genes. (D) Uroplakins are highly specific to the urothelium. Their loss after malignant transformation indicates impaired urothelial cytodifferentiation.



Annexin A10 (ANXA10) encodes a member of the annexin family of calcium-dependent phospholipid-binding protein. This protein was found to play a role in the regulation of cellular growth and in signal transduction pathways in BC [33]. The last gene in this panel, UPK1B, encodes a uroplakin. Four different uroplakin proteins are known at the present time. These heterodimerize and form urothelial plaques on the surface of urothelial cells. Uroplakins are significantly downregulated during urothelial transformation and tumorigenesis. In BC, UPK1B gene transcription is regulated epigenetically via CpG methylation [34].

In the present study, we were able to show the significantly higher overall sensitivity (84%) and NPV (93%) of the Xpert BC Monitor compared with bladder washing cytology (33% vs 76%), whereas the specificity of the two methods was similar (91% vs 94%). The sensitivity of the Xpert BC Monitor was high even in low-grade (77%) and Ta tumours (82%) compared with barbotage cytology (13% and 21%). All high-grade tumours were detected by the Xpert BC Monitor test, and only 83% by barbotage cytology. Our findings are in line with preliminary results presented by Van Valenberg et al. [35] at the EAU Congress 2017, who also investigated the impact of the Xpert BC Monitor in 255 patients undergoing BC surveillance. The authors registered an overall sensitivity, specificity and NPV of 75%, 81% and 94%, respectively, compared with 30%, 91% and 86%, respectively, for cytology [35].

The advantages of the Xpert BC Monitor are its automation, easy handling, its brief hands-on sample preparation time, single-use disposable cartridges, a GeneXpert instrument system that automates and integrates all complex PCR processes, and high-quality standards including in-sample quality controls. As the PCR cartridges are self-contained, cross-contamination between samples is kept to a minimum. In addition, RT-PCR-based urinary tests have the advantage of being reliable, easy to perform, and objective in contrast to cytology [29]. One of the major limitations of the use of mRNA-based techniques is the difficulty in obtaining a sufficient quantity of high-quality RNA from voided urine [28,29]. To overcome this problem, a positive ABL1 signal, indicating that the urine sample contains sufficient human cells and human RNA, was required for a valid test result (positive or negative) when using the Xpert BC Monitor. There is a wide variability, however, in the cost of mRNA-based urine tests (approximately €150 for the Xpert BC Monitor), so it needs to be more cost-effective in future for widespread use.

In conclusion, we established, for the first time, the superiority of the Xpert BC Monitor test over the gold standard in the follow-up and surveillance of patients with NMIBC. The Xpert BC Monitor provides an opportunity to improve the current standard of care. It has a significantly higher sensitivity and NPV than cytology, thus enhancing the efficacy and reducing the invasiveness of surveillance in patients with BC, including those with low-grade and pTa cancers. This encouraging hypothesis-generating study calls for further prospective randomized trials with sufficient statistical power before a final conclusion can be drawn.

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Conflict of Interest

None declared.

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Abbreviations: AUC, area under the curve; BC, bladder cancer; BTA, bladder tumour-associated antigen; CRH, corticotropin-releasing hormone; EAU, European Association of Urology; LDA, linear discriminant analysis; NMIBC, non-muscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver-operating characteristic.

