Xpert Bladder Cancer Monitor for Bladder Cancer Surveillance: First Experiences from a novel mRNA-based Urine Test

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Non-muscle invasive bladder cancer (NMIBC), with a recurrence rate in up to 70%, requires regular follow-up cystoscopies and cytologies. A validated urine marker would reduce the number of follow-up cystoscopies, but has not been established thus far. The aim of this study was to evaluate the diagnostic accuracy of the Xpert Bladder Cancer (BC) Monitor, compared to cystoscopy and cytology in the oncological follow-up of NMIBC[1].

MATERIAL and METHODS

- **140 patients** with a previous history of NMIBC undergoing routine surveillance at our department were enrolled prospectively (ISRCTN study registry number 37210907).
- **Urine cytology** was evaluated according to the Paris classification system. In addition, urinary specimens were analyzed using the Xpert BC Monitor, which measures **five target mRNAs** (ABL1, CRH, IGF2, UPK1B, ANXA10) using real-time-PCR. **External quality controls** (Xpert Bladder Cancer Panel C104, Maine Molecular Quality Controls, Inc., Saco, USA) were performed to monitor the performance of the Xpert BC Monitor, including low positive controls and negative controls.
- Descriptive analysis, diagnostic accuracy including sensitivities, specificities, predictive values [positive (PPV) and negative (NPV)], receiver operating characteristic (ROC) curves, and area under the curve (AUC) were calculated.

RESULTS

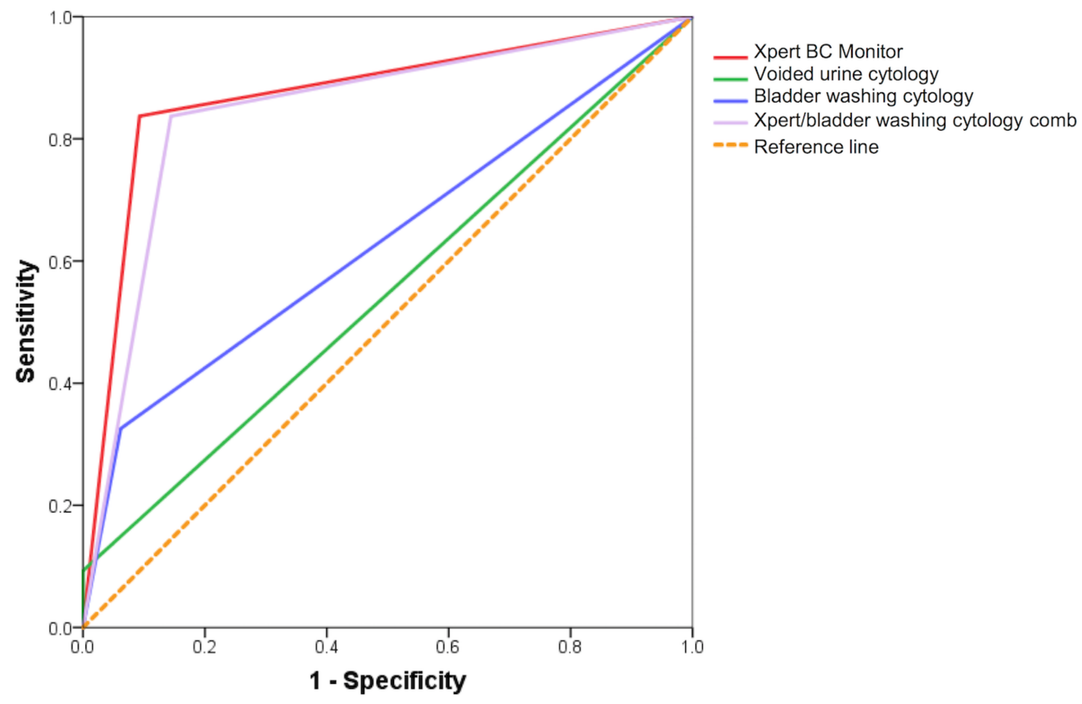
Urinary Test		True positives (TP)	True negatives (TN)	False positives (FP)	False negatives (FN)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Bladder washing cytology									
Overall		14	91	6	29	0.33 (0.19, 0.49)	0.94 (0.87, 0.98)	0.70 (0.46, 0.88)	0.76 (0.67, 0.83)
Gender	Female	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)
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)
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)	-	-	-
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)
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)
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)	-	-	-

Comparison of the diagnostic accuracy of bladder washing cytology, and the Xpert BC Monitor (overall and stratified by patient demographics and recurrent tumor classification).
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 showed a similar high **overall specificity** (91% *vs.* 94%, $p=0.41$).
While the sensitivity of the Xpert BC Monitor was 100% in patients with **high-grade tumors** compared to 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%) **tumors** (<3 cm) using the Xpert BC Monitor compared to bladder washing cytology, respectively.

Abbreviations: CI= confidence interval; FN= false negatives; FP= false positives; PPV= positive predictive value; NPV= negative predictive value; TN= true negatives; TP= true positives.

Diagnostic performances	Change sensitivity	Change specificity	Change PPV	Change 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*

Changes in sensitivity, specificity, PPV and NPV between the Xpert BC Monitor alone, voided urine cytology alone, bladder washing cytology alone, and combined (Xpert BC Monitor and bladder washing cytology) testing.
P-values are presented by McNemar`s test for sensitivity and specificity, and the generalized score statistic test by Leisenring *et al* for PPV and NPV. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.
Abbreviations: PPV= positive predictive value; NPV= negative predictive value;



Diagnostic test method	AUC (95% CI)	*P*-Value
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***

ROC curves and AUCs including 95% confidence intervals (95% CI) calculated for voided urine cytology, bladder washing cytology, the Xpert BC Monitor and the combination of the Xpert BC Monitor with bladder washing cytology.
The ROC curve analysis confirmed the diagnostic superiority of the Xpert BC Monitor (AUC=0.87, 95% CI: 0.80-0.94, $p<0.001$) over barbotage cytology (AUC=0.63, 95% CI: 0.53-0.74, $p=0.013$). Our data clearly show that combining the Xpert BC Monitor with barbotage cytology does not enhance diagnostic accuracy compared to Xpert BC Monitor measurement alone (the AUC for the combination was 0.85 versus 0.87 for the Xpert BC Monitor alone).
P values by the Mann-Whitney-U test; *$p<0.05$; **$p<0.01$; ***$p<0.001$.
Abbreviations: AUC= area under the curve; CI= confidence interval;

CONCLUSIONS

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

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(1) Pichler R, Fritz J, Tulchiner G, Klinglmair G, Soleiman A, Horninger W, Klocker H, Heidegger I. *Increased Accuracy of a novel mRNA-based Urine Test for Bladder Cancer Surveillance*. **BJU Int**. Accepted online, 11 Sep 2017