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Xpert® bladder cancer detection as a diagnostic tool in upper urinary tract urothelial carcinoma: preliminary results

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Abstract

Objectives: Upper urinary tract urothelial carcinoma (UTUC) represents about 5–10% of all urothelial malignancies with an increasing incidence. The standard diagnostic tools for the detection of UTUC are cytology, computed tomography (CT) urography, and ureterorenoscopy (URS). No biomarker to be included in the daily clinical practice has yet been identified. The aim of our study was to evaluate the potential role of Xpert® Bladder-Cancer (BC)-Detection in the diagnosis of UTUC.

Methods: Eighty-two patients underwent 111 URS with Xpert® BC-Detection, cytology, or Urovysion® analysis of UT for suspicion of UTUC. Twenty-four cases were excluded from the analysis due to a non-diagnostic Xpert® BC-Detection, cytology, or Urovysion®. Samples were analyzed with upper tract (UT) urinary cytology, with Xpert® BC-Detection on UT urines, and with Urovysion® Fluorescence *in situ* hybridization (FISH) test. After urine collection, the patients underwent retrograde pyelography and/or URS, and if positive a UT biopsy. The Xpert® BC-Detection was reported by the software as negative or positive [cut-off total Linear Discriminant Analysis (LDA) = 0.45]. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cytology, Xpert® BC-Detection and Urovysion-FISH were calculated using URS and/or histology results as reference.

Results: In all, 27 (31%) of 87 URS resulted positive, with 20 low-grade (LG) and 7 high-grade (HG) tumors. Overall sensitivity was 51.9% for cytology, 100% for Xpert® BC-Detection, and 92.6% for Urovysion. The sensitivity of cytology increased from 26% in LG to 100% in HG tumors. For Xpert® BC-Detection, sensitivity was 100% both in LG and in HG, and for Urovysion-FISH, it increased from 90% in LG to 100% in HG tumors. PPV was 82.4% for cytology, 35% for Xpert® BC-Detection, and 73.5% for Urovysion. NPV was 81.4% for cytology, 100% for Xpert® BC-Detection, and 96.2% for Urovysion.

Conclusion: The excellent NPV of Xpert® BC-Detection allows to avoid unnecessary endoscopic exploration of the UT, reducing invasiveness and URS complications in the follow-up of UTUC.

Keywords: upper urinary tract, ureterorenoscopy, urinary markers, urothelial carcinoma

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Introduction

Urothelial cancer (UC) can affect the bladder, ureter, renal pelvis or calix, and urethra.[1] Upper urinary tract urothelial carcinoma (UTUC) is not uncommon in Western countries with about 150,350 new cases and 33,170 estimated deaths in the United States in 2018 in both sexes.

The renal pelvis seems to be the most frequent location (64%), as documented by Margulis *et al.* in a series of 1363 patients treated with nephroureterectomy at 12 academic centers. About 63% of the patients showed a high-grade (HG) disease and more than 55% were invasive at diagnosis.[2] UTUC are often silent, and due to the diagnostic delay in more than 50% of cases, they are often diagnosed in an advanced stage.

The aggressiveness of this disease seems to be increasing in the last years. Lughezzani *et al.* evaluated 4915 patients with UTUC between 1983 and 2004 and treated them with a nephroureterectomy or a segmental ureterectomy. The analysis of the data evidenced an increase of non-localized stage from 49.8% to 69.5% ($p < 0.001$) and of grade III–IV from 45.7% to 70.2% ($p < 0.001$) between 1983 and 2004. However, an increase in cancer-specific mortality was not observed, which also confirms the effectiveness of surgical treatment.[3]

According to these data, it is essential to perform a rapid and prompt diagnosis. Following the current European Association of Urology (EAU) guidelines, diagnosis and staging can be performed with computed tomography (CT) urography and flexible ureterorenoscopy (URS).[4]

In a meta-analysis from Janisch *et al.*,[5] the pooled sensitivity and specificity of CT urography for UTUC were 92% and 95%, respectively; however, it cannot detect flat lesions and its systematic use can represent a significant economic burden, also requiring specialized centers. Flexible URS allows identification of a tumor in more than 90% of cases and to take a diagnostic biopsy, even if an under-grading and under-staging of the tumor can occur.[6]

During URS, urine samples for cytology can be collected directly from the renal pelvis or ureter. Urinary cytology may play a role in the UTUC diagnosis. An abnormal voided urinary cytology and negative cystoscopy may suggest the presence of a UTUC. While upper tract (UT) cytology can add a useful information for diagnosis and risk stratification of the tumor,[4] URS remains the gold standard for diagnosis and surveillance of UTUC. However, URS is an invasive procedure and is burdened with serious complications, such as ureteral perforation, bleeding, or infections.[7] There is, therefore, a need for reliable markers to improve detection of UTUC and to reduce surgical follow-up with procedure-associated complications.

Currently, no marker is recommended by the EAU guidelines for diagnosis or follow-up of UTUC since to date no one has shown adequate performance.

This study evaluates the performance and the clinical utility of a new mRNA-based urinary marker, Xpert® BC-Detection, in the detection of UTUC and compares it with cytology and the Urovysion® Fluorescence *in situ* hybridization (FISH) test.

Material and methods

After approval of the local institutional ethics committee (Ethics Committee of General Hospital of Bolzano, study registration number: 10-2018) and after written and oral informed consent of the patients, 82 patients undergoing URS for suspicion of UTUC were included in our single-center prospective study.

Samples were analyzed with UT urinary cytology, Xpert® BC-Detection, and Urovysion®-FISH; patients underwent URS under general anesthesia and, if positive, a UT biopsy. Any suspicious lesion was biopsied or removed and specimens were evaluated according to the 2017 TNM classification of UTUC and graded according to both the 1973 and the 2004 World Health Organization (WHO) grade classification.

The Xpert® BC-Detection was reported by the software as negative or positive (cut-off total LDA = 0.45). LDA is the Linear Discriminant Analysis, a score that provides a 'negative' or 'positive' result according to a cut-off defined by the manufacturer. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cytology, Xpert® BC-Detection, and Urovysion®-FISH were calculated using URS and/or histology results as reference.

We collected UT urine directly from renal pelvis or ureter with a ureteral catheter during URS with the aim to obtain 4–5 ml of UT urine for each sample. The UT urine was added to the Xpert® Urine Transport Reagent Kit, an RNA stabilizing reagent. The residual urine was added to 15 ml of Cytolyt fixation liquid (Hologic, Inc., Marlborough, MA) in a Falcon tube and used for cytology and Urovysion®-FISH test.

Xpert® BC-detection

The Xpert® BC-Detection, performed on Cepheid GeneXpert® Instrument Systems, is a non-invasive qualitative *in vitro* diagnostic test created for early diagnosis in patients with suspicion of UC. This test measures the level of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) in 4.5 ml urine sample using real-time, reverse transcription polymerase chain reaction (RT-PCR) in a prefilled cartridge.

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithm in about 90 min. The test result, LDA totals, and analyte results are shown on the test report. The cut-off for positive test result is set at an LDA of >0.45. The test is easy to perform and does not need a professional technician.

In this study, the test has been used off-label with the same cut-off value as for voided urine, to detect the presence of UTUC.

Cytology

The tubes were centrifuged for 10 min at 800*g*. The resulting cell pellets were re-suspended in ThinPrep vials containing PreservCyt solution and processed by the TP 5000 System (Hologic).

Cytological evaluation was performed using the Papanicolaou staining procedure and the Paris System for Reporting Urinary Cytology.8 For the statistical analysis, NHGUC (negative for High-Grade Urothelial Carcinoma) and AUC (Atypical Urothelial Cells) were grouped as negative and SHGUC (Suspicious for High-Grade Urothelial Carcinoma), HGUC (High-Grade Urothelial Carcinoma), and LGUN (Low-Grade Urothelial Carcinoma) as positive.

A second slide was prepared to be used for the Urovysion®-FISH.

Cytology is a cheap and quick test, easy to perform, but it is highly dependent on the examiner and needs a dedicated cytopathologist.

Urovysion®-FISH test

Multicolour-FISH was performed on liquid-based urinary cytology using the Urovysion® Bladder Cancer Kit (Abbott Molecular, Des Plaines, IL). Slides were scored for hybridization signals on a cell-by-cell basis, using an Olympus Provis BX61 (Olympus Italia, Milan, Italy) with a filter set including diamidino phenylindole single bandpass (counterstain), aqua single bandpass (chromosome 17), gold single bandpass (9p21 locus), and a red–green double bandpass (chromosomes 3 and 7). Enumeration and evaluation of the FISH signals were carried out on target cells that appeared morphologically abnormal, according to Bubendorf *et al.*[9]

Statistical analysis

The sensitivity, specificity, PPV, and NPV of cytology, Xpert® BC-Detection, Urovysion®-FISH test, and their combination were calculated and compared with retrograde pyelography/URS/histology.

Results

In all, 130 analyses were performed in 82 patients with a mean age of 69.9 years (SD: 11.6). Twenty-four cases were excluded from the analysis due to a non-diagnostic Xpert® BC-Detection in 5 cases (3.8%), non-diagnostic cytology in 8 cases (6.1%), and non-diagnostic Urovysion® in 11 cases (8.5%).

Other 19 analyses were excluded because in these cases we performed only a pyelography without diagnostic URS.

A total of 87 analyses were evaluable and included in the study. In all, 27 (31%) of 87 URS resulted positive, with 20 LG (74%) and 7 HG (26%) tumors (Table 1).

Overall sensitivity was 100% for Xpert® BC-Detection, 51.9% for cytology, and 92.6% for Urovysion®-FISH test. The sensitivity of Xpert® BC-Detection was 100% in both LG and HG tumors, and the sensitivity of cytology increased from 26% in LG to 100% in HG tumors and of Urovysion®-FISH from 90% in LG to 100% in HG tumors.

The specificity was 16.7% for Xpert® BC-Detection, 95% for cytology, and 85% for Urovysion®-FISH. PPV was 35% for Xpert® BC-Detection, 82.4% for cytology, and 73.5% for Urovysion®, and NPV was 100% for Xpert® BC-Detection, 81.4% for cytology, and 96.2% for Urovysion® (Table 2).

Table 1. Baseline characteristics of the patients.

Age at the procedure	
Mean (years)	69 + 12.6
Median (years)	70.5
Gender	
F	17
M	50
Procedures	106
URS	87
Pyelography	19
Tumors	27
LG	20
HG	7
First diagnosis (pts)	11

HG, high-grade; LG, low-grade; URS, ureterorenoscopy.

Table 2. Sensitivity, specificity, and predictive values of cytology, Xpert® BC-Detection, and Urovysion®-FISH in 87 analyses.

	Xpert® BC-Detection	**Cytology**	**Urovysion®-FISH**
Sensitivity	100%	51.9%	92.6%
Specificity	16.7%	95%	85%
PPV	35%	82.4%	73.5%
NPV	100%	81.4%	96.2%

BC, bladder cancer; FISH, fluorescence *in situ* hybridization; NPV, negative predictive value; PPV, positive predictive value.

All cases with negative Xpert® BC-Detection had negative cytology too.

The AUC for Xpert was 0.63 (52.0–75.9 IC).

Discussion

Urothelial carcinoma is the fourth most frequent tumor in the developed countries; UTUC represents about 5–10% of all urothelial carcinomas, with pyelocaliceal localization twice more frequent compared with ureteral localization.[1] In contrast to bladder cancer, UTUC is more frequently invasive at diagnosis;[2] it is therefore mandatory to get an early diagnosis to improve cancer outcomes.

According to the EAU guidelines, the diagnostic tools for the detection of UTUC are URO CT (or MRI) and URS with biopsies. No biomarker test has been yet included in the routine clinical practice.

Xpert® BC-Detection is a non-invasive urinary test intended to detect the presence of bladder cancer. The aim of our study was to evaluate the performance of this test in patients with suspicion of UTUC.

In our series, overall sensitivity was 100% for Xpert® BC-Detection, 51.9% for cytology, and 92.6% for Urovysion®-FISH. Specificity was 16.7% for Xpert® BC-Detection, 95% for cytology, and 85% for Urovysion®-FISH.

At the moment, there are no data regarding the performance of Xpert® BC-Detection in the diagnosis of UTUC.

In 2014, Monteiro Res *et al.* evaluated the performance of a panel of three epigenetic biomarkers (promoter methylation of GDF15, TMEFF2, and VIM) on 57 UTUC tissues, 36 normal UT urothelium, 22 voided urines from UTUC suspects, and 20 urines from controls, reporting a sensitivity of 91% and specificity of 100% and an AUC of 0.923 of the panel on urinary samples.

By comparison, sensitivity of the Xpert® BC-Detection on UT urine in our cohort was higher (100%). In contrast, specificity and PPV were significantly lower in our study (16.7% and 35%) than reported by Monteiro-Reis *et al.* (100%).[10] NPV was lower in Monteiro's study (91%)[10] than in our cohort (100%).

More recently, Boissier *et al.* conducted a single-center prospective non-comparative study including 25 patients with a suspicion of uni- or bilateral UTUC on the diagnostic accuracy of the Bladder Epicheck® kit, a newly developed urinary marker based on DNA methylation changes associated with UC in a panel of 15 genomic biomarkers. Overall, sensitivity of Bladder Epicheck® was 67% on UT urine with 89% sensitivity in HG UTUC,[11] substantially lower than in our study.

They reported a specificity of 85%, performing substantially better than Xpert® Bladder Detection on UT urine (13.9%).

Messer *et al.* analyzed retrospectively 326 patients with clinically localized UTUC who had undergone radical nephroureterectomy or distal ureterectomy. In the subgroup of patients with available cytology from selective ureteral catheterization, sensitivity was 71% for detecting HG disease and 78% for muscle-invasive UTUC[12] in contrast to our study, where cytology showed an overall sensitivity of 51.9%, reaching 100% in HG tumors, with an excellent specificity (95%).

In 2001, Lodde *et al.*[13] evaluated the performance of cytology and Immuno-Cyt in UTUC diagnosis, showing a sensitivity on the voided urine of 50% for cytologic analysis, 75% for Immuno-Cyt, and 87% for both methods combined. However, Immuno-Cyt is no longer on sale.

In 2010, 55 consecutive patients with a suspected UTUC were studied with intravenous pyelography, cytology, washing cytology, Urovysion®-FISH, and URS by Mian *et al.*[14] The sensitivity of cytology was 20.8%, lower than in the present study (51.9%), but the sensitivity of Urovysion®-FISH was 100%, performing significantly better (92.6%); specificity was similar: 97.4% for cytology and 89.5% for Urovysion-FISH® *versus* 95% and 85%, respectively.

The performance of Xpert® BC-Detection could not reach the performance of Urovysion in UTUC diagnosis, but its high NPV is of clinical relevance since it allows to reduce invasive, potentially risky, and cost-intensive investigation; if negative, it could support a less invasive follow-up.

However, this study has some limitations: we used a marker, actually validated only for bladder cancer detection, which is based on a cut-off established in a training set from voided urine; this cut-off could not be applicable to samples collected invasively: because of disruption of the urothelium in the process of collecting the sample, we may see a higher false-positive rate and could require a higher cutoff-value. However, a potential predictive value of false-positive cases needs to be evaluated during follow-up.

The relatively small number of patients with UTUC, that is, a rather uncommon neoplasm, needs a validation in larger multicenter studies, possibly in the context of well-designed randomized prospective trials.

Conclusion

Manipulation of the UT seems to decrease the specificity of the Xpert® BC-Detection, limiting the usefulness in the diagnosis of UTUC. However, an excellent NPV of clinical relevance allows to avoid unnecessary endoscopic exploration of the UT, reducing the invasiveness and the burden of URS complications in the follow-up of UTUC.

Authors' note

*The views and opinions expressed in this article are those of the authors and do not necessarily reflect the position of the organization of which they are part.

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Conflict of interest statement

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References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics 2019. *CA Cancer J Clin* 2019; 69: 7–34.
2. Margulis M, Shariat SF, Matin SF, *et al.* Outcomes of radical nephroureterectomy: a series from the Upper Tract Urothelial Carcinoma Collaboration. *Cancer* 2009; 115: 1224.
3. Lughezzani G, IsbarnJeldres CH, Isbarn H, *et al.* Temporal stage and grade migration in surgically treated patients with upper tract urothelial carcinoma. *BJU Int* 2010; 105: 799–804.
4. Rouprêt M, Babjuk M, Burger M, *et al.* *EAU guidelines on upper urinary tract urothelial carcinoma*. European Association of Urology, March 2020, http://uroweb.org/guidelines/compilations-of-all-guidelines/
5. Janisch F, Shariat SF, Baltzer P, *et al.* Diagnostic performance of multidetector computed tomographic (MDCTU) in upper tract urothelial carcinoma (UTUC): a systematic review and meta-analysis. *World J Urol* 2020; 38: 1165–1175.
6. Smith AK, Stephenson AJ, Lane BR, *et al.* Inadequacy of biopsy for diagnosis of upper tract urothelial carcinoma: implications for conservative management. *Urology* 2011; 78: 82–86.
7. Schoenthaler M, Buchholz N, Farin E, *et al.* The Post-Ureteroscopic Lesion Scale (PULS): a multicentric video-based evaluation of inter-rater reliability. *World J Urol* 2014; 32: 1033–1040.
8. Rosenthal DL, Wojcik EM, Kurtycz DFI, *et al.* *The Paris system for reporting urinary cytology*. Cham: Springer International Publishing, 2016.
9. Bubendorf L, Grilli B, Sauter G, *et al.* Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. *Am J Clin Pathol* 2001; 116: 79–86.
10. Monteiro-Reis S, Leça L, Almeida M, *et al.* Accurate detection of upper tract urothelial carcinoma in tissue and urine by means of quantitative GDF15, TMEFF2 and VIM promoter methylation. *Eur J Cancer* 2014; 50: 226–233.
11. Boissier DR, Territo A, Gallioli A, *et al.* Feasibility of the Epicheck test in upper urinary tract tumour: interim analysis of a prospective trial. *SC318 – EAU 2020 virtual*, 17–20 July, https://nucleix.com>uploads>2020/07>9
12. Messer J, Shariat SF, Brien JC, *et al.* Urinary cytology has a poor performance for predicting invasive or high-grade upper-tract urothelial carcinoma. *BJU Int* 2011; 108: 701–705.
13. Lodde M, Mian C, Wiener H, *et al.* Detection of upper urinary tract transitional cell carcinoma with ImmunoCyt: a preliminary report. *Urology* 2001; 58: 362–366.
14. Mian C, Mazzoleni G, Vikoler S, *et al.* Fluorescence in situ hybridisation in the diagnosis of upper urinary tract tumours. *Eur Urol* 2010; 58: 288–292.