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UBC® *Rapid* Test for detection of carcinoma in situ for bladder cancer

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Abstract

UBC® *Rapid* Test is a test that detects fragments of cytokeratins 8 and 18 in urine. We present results of a multicentre study measuring UBC® *Rapid* Test in bladder cancer patients and healthy controls with focus on carcinoma in situ (CIS) and high-grade bladder cancer. From our study with N = 452 patients, we made a stratified sub-analysis for carcinoma in situ of the urinary bladder. Clinical urine samples were used from 87 patients with tumours of the urinary bladder (23 carcinoma in situ, 23 non-muscle-invasive low-grade tumours, 21 non-muscle-invasive high-grade tumours and 20 muscle-invasive high-grade tumours) and from 22 healthy controls. The cut-off value was defined at 10.0 µg/L. Urine samples were analysed by the UBC® *Rapid* Test point-of-care system (concile Omega 100 POC reader). Pathological levels of UBC *Rapid* Test in urine are higher in patients with bladder cancer in comparison to the control group ($p < 0.001$). Sensitivity was calculated at 86.9% for carcinoma in situ, 30.4% for non-muscle-invasive low-grade bladder cancer, 71.4% for nonmuscle-invasive high grade bladder cancer and 60% for muscle-invasive high-grade bladder cancer, and specificity was 90.9%. The area under the curve of the quantitative UBC® *Rapid* Test using the optimal threshold obtained by receiveroperated curve analysis was 0.75. Pathological values of UBC® *Rapid* Test in urine are higher in patients with high-grade bladder cancer in comparison to low-grade tumours and the healthy control group. UBC® *Rapid* Test has potential to be more sensitive and specific urinary protein biomarker for accurate detection of high-grade patients and could be added especially in the diagnostics for carcinoma in situ and non-muscle-invasive high-grade tumours of urinary bladder cancer.

Keywords

UBC® *Rapid* Test, bladder cancer, carcinoma in situ, urinary tumor marker

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Introduction

Urinary bladder cancer has a high rate of recurrence; a substantial number of non-invasive tumours will progress to muscle-invasive disease. Especially, carcinoma in situ (CIS) is a very aggressive form of urinary bladder cancer, almost high grade and thought to be the most common precursor of invasive tumours.[1] CIS was first reported by Melicow.[2] Despite the non-invasive character of CIS, it was suspected to possess aggressive tumour biology, and tendencies towards early progression were described.[3] CIS is rare, and it is found in approximately 3% of the cases.[4]

In addition, CIS is difficult to detect. Even cystoscopy being the gold standard for bladder cancer detection, it is not easy to find,[5] and classic white-light cystoscopy (WLC) can miss up to 50% of cases.[6] Urothelial CIS is a flat lesion characterized by the presence of unequivocal cytologically

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malignant cells.[1] Extensive denudation of the urothelium, monomorphic appearance of the neoplastic cells, inflammatory atypia, radiation-induced nuclear smudging, multinucleation and pagetoid spread of CIS may cause diagnostic difficulties. Together with clinical and morphologic correlation, immunostaining with CK20, p53 (full thickness) and CD44 (absence of staining) may help accurately diagnose CIS.[7] Urothelial CIS with glandular differentiation or pagetoid changes is a variant of CIS that follows the natural history of conventional urothelial CIS.[8–10]

Traditionally, the detection of CIS was performed with a combination of urine cytology, cystoscopy and multiple bladder biopsies.[11] Urine cytology has a calculated sensitivity for any form of CIS of approximately 60%.[12] Within the limitations of random sampling and pathologic assessment, multiple bladder biopsies have a sensitivity of approximately 77%.[13]

Urine soluble markers should be able to ensure primary diagnosis, follow-up control and screening of high-risk populations. These urinary-based assays may detect the presence of bladder cancer, because the disease is in contact with urine constantly, malignant cells are shed into the urine, and it is likely that urine contains the carcinogens producing the malignancy. Nowadays, there are other urine-based possibilities for bladder cancer detection. Some of these methods have a higher specificity and sensitivity than classical urine cytology and can be important for screening.[14]

As intermediate filaments of the cytoskeleton, cytokeratins are to enable cells to withstand mechanical stress. In humans, 20 different cytokeratins have been identified, and cytokeratins 8, 18, 19 and 20 are known as important in bladder cancer.[15] Immunohistochemical features of urothelial dysplasia include aberrant cytokeratin 20 expression at different levels of the urothelium, but also, there is usually overexpression of p53 and high Ki-67 index.[16] Cytokeratin 20 can be measured in higher levels in tumours. The expressions of cytokeratins such as cytokeratins 8, 18 and 19 are higher in urothelial cells and may be elevated because of a higher cell turnover rate.[17,18]

UBC® *Rapid* Test can measure fragments of cytokeratins 8 and 18 qualitatively. The measured levels are lower in low-grade tumours and benign urological diseases.[19,20] Cytokeratins 8 and 18 are soluble in urine and can be detected quantitatively with monoclonal antibodies using sandwich enzyme-linked immunosorbent assay (ELISA). The aim of this multicentre study was to evaluate the usefulness of UBC *Rapid* Test in patients with CIS of urinary bladder cancer comparing with healthy individuals.

Materials and methods

Patients

For this prospective study, 452 urine samples from bladder cancer patients and healthy controls have been collected between January 2014 and October 2015 at the Department of Urology, HELIOS Hospital Bad Saarow (study centre I) and Lukas Hospital Neuss (study centre II), Germany. The study was approved by the local Institutional Review Board of National Medical Association Brandenburg. All patients with confirmed bladder cancer underwent cystoscopy, bladder ultrasound and transurethral resection of bladder tumour in case of abnormal findings. Exclusion criteria were any kind of mechanical manipulation (cystoscopy, transrectal ultrasound and catheterization) within 10 days before urine sampling. Other exclusion criteria were benign prostate enlargement, urolithiasis, other tumour diseases, severe infections and pregnancy. All these criteria could influence the test to false-positive results. This is the highest number of samples measured for UBC *Rapid* Test; therefore, it was possible to make a subgroup analysis with focus on CIS.

From this primary cohort of 452 urine samples, we made a stratified sub-analysis for CIS of urinary bladder cancer. Therefore, clinical urine samples were used from 87 patients with urinary bladder cancer (23 CIS, 23 non-muscle-invasive low-grade (NMI-LG) tumours, 21 non-muscle-invasive high-grade (NMI-HG) tumours and 20 muscle-invasive high-grade (MI-HG) tumours) and from 22 healthy controls. All patients were paired matched in age and gender.

Procedure

Midstream urine was collected in a sterile plastic container and processed subsequently. Urine samples were analysed by the UBC *Rapid* Test (concile GmbH, Freiburg/Breisgau, Germany). All tests were carried out as advised by the manufacturer's instructions. The presence of a test band after 10 min of incubation was checked. After visual evaluation, the test cartridges were analysed by the photometric point-of-care (POC) system concile Omega 100 reader (concile GmbH, Freiburg/Breisgau, Germany) for quantitative analysis. The cut-off value was defined at 10 µg/L. The Omega 100 reader illuminates the test field with a complementary coloured light to reduce interference in the analysis. The built-in charge-coupled device–matrix sensor takes a photograph of the light reflected, which is analysed by the device.

Statistical analysis

The predictive ability of UBC *Rapid* Test to detect urinary bladder cancer was analysed by means of receiver operating characteristic (ROC) analysis. The optimal cut-point is defined at the point that maximizes the Youden index. The sensitivity, specificity, and negative predictive value (NPV) and positive predictive value (PPV) are presented at the optimal cut-off for each analysis.

All statistical analyses were performed using R version 3.2.3.[21]

Table 1. Patient characteristics and results of UBC® *Rapid* Test.

	CIS	NMI-LG	NMI-HG	MI-HG	Control	Control-reference	p value
n	23	23	21	20	22	146	
Age (years)							0.501
Mean (SD)	72.22 (6.85)	71.48 (6.89)	73.05 (8.40)	71.55 (8.95)	71.86 (7.11)	67.41 (12.76)	
Median	74	72	74	74.5	73	69	
Range	57–81	57–84	57–89	52–83	57–84	31–86	
Sex							0.608
Female (%)	7 (30.43)	7 (30.43)	5 (23.81)	5 (25.00)	7 (31.82)	57 (39.04)	
Male (%)	16 (69.57)	16 (69.57)	16 (76.19)	15 (75.00)	15 (68.18)	89 (60.69)	
UBC *Rapid* Test							<0.001
Mean (SD)	66.0 (81.0)	12.2 (19.1)	89.3 (102.1)	60.7 (91.7)	5.9 (3.1)	7.4 (10.2)	
Median	23.4	5.2	42	20.45	5	5	
Range	5–300	5–84.4	5–300	5–300	5–18.8	5–90.9	
Sensitivity	86.9%	30.4%	71.4%	60%	–	–	
Specificity	–	–	–	–	90.9%	93.8%	

NMI-LG: non-muscle-invasive low grade; NMI-HG: non-muscle-invasive high grade; MI-HG: muscle-invasive high grade; SD: standard deviation.

Results

In the stratified sub-analysis for this project, a total of 109 patients were included in the study, 87 with confirmed bladder cancer and 22 healthy controls with no history of bladder cancer. The median age of the study population was 72 (range = 52–89) years. Of these patients, 78 (71.6%) were men and 31 (28.4%) were women.

We could show that pathological concentrations of UBC *Rapid* Test are detectable in urine of bladder cancer patients. The characteristics for age and sex as well as the results of UBC *Rapid* Test for bladder cancer patients and healthy controls are listed in Table 1. Both study centres enrolled a similar number of patients in the study.

Due to the focus on CIS and high-grade bladder cancer patients, we decided not to compare with cytology and other urinary markers in that clinical setting. In our CIS cohort, we have only one isolated CIS, and others are CIS in combination with other pathologies such as pTa, pT1 and pT2+.

Pathological levels of UBC *Rapid* Test in urine are higher in patients with bladder cancer in comparison to the control group. In 23 CIS, the mean value of UBC *Rapid* Test was 66.0 µg/L, for NMI-LG tumours 12.2 µg/L, for NMI-HG tumours 89.3 µg/L, for MI-HG tumours 60.7 µg/L and for the healthy individuals 5.9 µg/L. Pathological levels of UBC *Rapid* Test in urine are statistically significantly higher in patients with bladder cancer in comparison to the control group ($p < 0.0001$). The area under the curve (AUC) of the quantitative UBC *Rapid* Test using the optimal threshold obtained by receiver-operated curve (ROC) analysis (cut-off = 10.0 µg/L) was 0.75 as shown in Figure 1. Figure 2 shows ROC analysis separated for the different risk groups: The calculated AUC is 0.918 for CIS at a cut-off value of 10.70 µg/L, 0.666 for NMI-LG at a cut-off value of 5.10 µg/L, 0.88 for NMI-HG at a cut-off value of 16.4 µg/L and 0.861 for MI-HG at a cut-off value of 6.40 µg/L. These cut-off values are the outcome after ROC optimization of the various cohorts. For the whole study group, we decided to take a cut-off value set on 10.0 µg/L for this study.

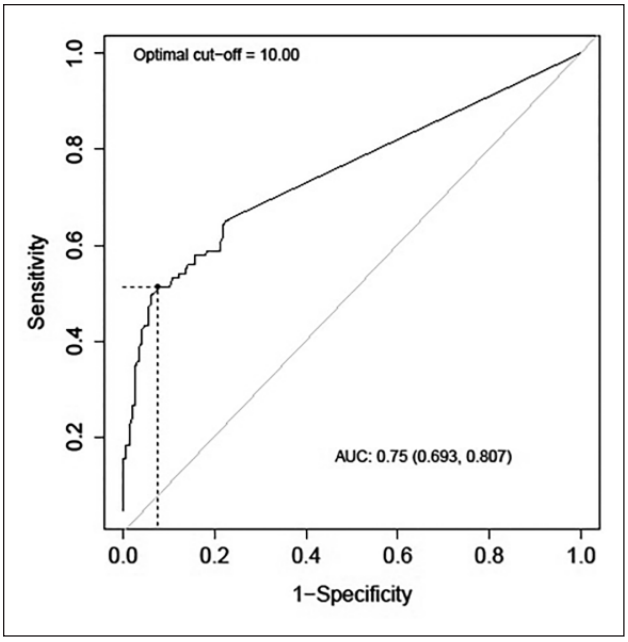

Figure 1. Analysis of the predictive ability – ROC curve analysis for UBC® *Rapid* Test at a cut-off value of 10.0 µg/L with AUC 0.75 for the whole population.

Box plot graph in Figure 3 shows the variation of values in the different tumour groups. It shows that most of the pathological values are higher than the cut-off especially for CIS and high-grade tumours.

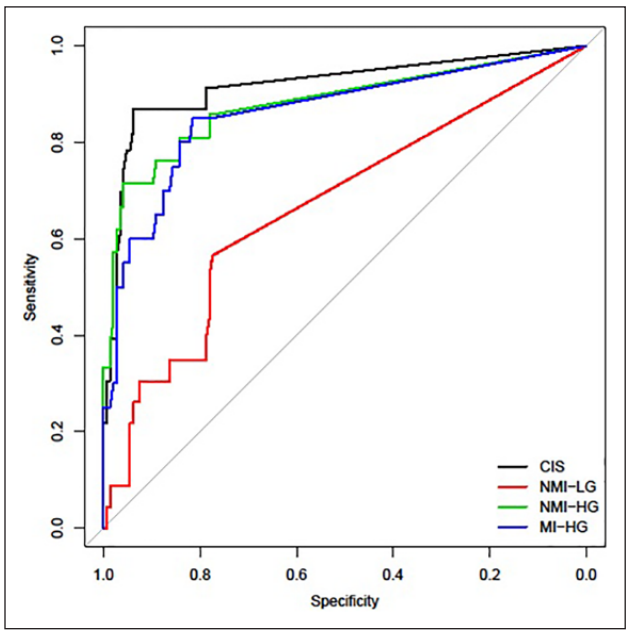

Figure 2. Analysis of the predictive ability – ROC curve analysis for UBC® *Rapid* Test at a cut-off value of 10.0 µg/L for all-risk groups: CIS (black), NMI-LG (red), NMI-HG (green), and MI-HG (blue).

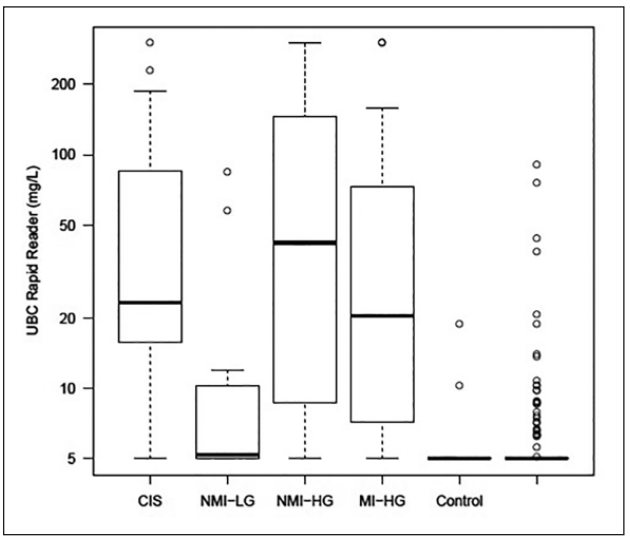

Figure 3. Box plot for CIS, non-muscle-invasive low grade (NMI-LG), non-muscle-invasive high grade (NMI-HG), muscle-invasive high grade (MI-HG), and control (matched group; all).

Table 2. Sensitivity, specificity, positive predictive value and negative predictive values using cut-off value 10.0 µg/L.

	Estimate	95% CI
Sensitivity	51.38%	41.61%–61.06%
Specificity	92.47%	86.92%–96.18%
PPV	83.58%	72.52%–91.51%
NPV	71.81%	64.80%–78.11%

PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval.

Sensitivity was calculated as 86.9% for CIS, 30.4% for NMI-LG tumours, 71.4% for NMI-HG tumours and 60% for MI-HG bladder cancer, and specificity was 90.9%. A specificity of 90.9% was calculated for the age- and sex-selected control group, and for the whole number of healthy individuals (n = 146) from the primary cohort, a specificity of 93.8% was calculated. Data of sensitivity, specificity, PPV and NPV using cut-off of 10.0 µg/L for UBC *Rapid* Test including the 95% confidence interval are listed in Table 2.

Discussion

Current guidelines recommend the use of urine markers only as an adjunct to cystoscopy owing to their limited accuracy.[22–24] Newer tests, such as fluorescence in situ hybridization (FISH) and immunocytology, have shown improved sensitivity compared with cytology,[25–27] but they are complex to perform and require specialized laboratory facilities. POC tests for bladder cancer have been introduced, aiming to overcome complex testing and high costs, and do provide a cost- and time-effective adjunct to cytology. The main limitations of most of these tests are their relatively high rate of false-positive tests (due to infection, mechanical manipulation, other tumour diseases, diabetes mellitus and the presence of stones).

The aim of this multicentre study was to evaluate the usefulness of UBC *Rapid* Test with special focus on patients with CIS and high-grade tumours of urinary bladder comparing with healthy individuals. The results of this study show that cytokeratin concentrations determined by the POC reader significantly correlated between patients with bladder cancer and healthy controls. Values of UBC *Rapid* Test in high-grade tumours are significantly higher than in low-grade tumours and healthy individuals. The AUC as a parameter of diagnostic quality of the quantitative UBC *Rapid* Test was calculated with 0.75 based on a cut-off value of 10.0 µg/L. UBC *Rapid* Test determined quantitatively not only the risk of bladder cancer in general increase but also the risk of having a high-grade tumour (G3, CIS) increased with higher test values. This feature underlines the significance of a quantitative consideration of UBC *Rapid* Test, as is also the case for other quantitative urinary markers.[28] Comparing with already published results, these new results show again high values for UBC *Rapid* Test for high-grade bladder cancer patients.[20,29] After one of the first UBC *Rapid* studies with a high number of samples more than 15 years ago,[30] our results now show that this test could be very useful to be combined in diagnostic for the high-risk group of bladder cancer patients. In former reports of UBC *Rapid* Test, a sensitivity of 65% and specificity of 92% were calculated.[30,31] Mian et al.[31] used an older

version for the measurement of UBC *Rapid* Test, and only visual evaluation was possible. In our study, an updated version of the cytokeratin assay for UBC *Rapid* Test was used; an improved lateral flow results in more clear test and control bands to evaluate. Furthermore, in our study, UBC *Rapid* Test is used in combination with the Omega 100 reader to quantify the results.

For sure, this was the beginning and first results of UBC *Rapid* Test, but newer studies reported at a cut-off of 12.3 µg/L a sensitivity, specificity, PPV, and NPV of 60.7%, 70.1%, 46.8%, and 79.3%, respectively, with an AUC of 0.68.[20]

After the European Association of Urology (EAU) guidelines, the examination of voided urine for exfoliated cancer cells has high sensitivity in high-grade tumours and is a useful indicator in cases of high-grade malignancy or CIS.[32] But there are also limitations for cytology. Evaluation of cytology specimens can be hampered by low cellular yield, urinary tract infections (UTIs), stones, or intravesical instillations, but for experienced readers, specificity exceeds 90%.[17,33] However, negative cytology does not exclude a tumour. Compared to the subjective method of cytology, the advantage of UBC *Rapid* Test is an objective method that is standardized and reproducible. Anyway, there is no known urinary marker specific for the diagnosis of invasive bladder cancer.[34]

It is common that new urine tests are compared with the results of cytology. In the large number of studies, the results vary a lot. Sensitivity for G1 tumours is lower than 30%, for G2 tumours around 60%, and for G3 tumours around 90%. Specificity is around 90%–95%.[35] In a prospective study, Schmitz-Dräger et al. compared immunocytology in patients with haematuria.[36] Therefore, 301 consecutive patients with haematuria and without tumour in patients' history have been tested with a commercial urine test. In 10 out of 228 patients (4.6%) with microhaematuria and 17 out of 66 patients (27%) with gross haematuria, bladder cancer could be detected. Patients with microhaematuria and bladder cancer showed sensitivity and specificity of 40% and 97% in cytology and 80% and 89% in immunocytology, respectively. Cystoscopy alone showed 80% and 99%. A combination of these tests could raise sensitivity by the same specificity. In the study by Ritter et al.,[20] UBC *Rapid* Test was also compared with cytology showing better results for UBC *Rapid* Test. Though it is one limitation of our study, this is one reason why we had no comparison to cytology due to the focus on high-grade urinary bladder cancer.

Regarding FISH as a tool for enhanced detection of CIS, it detects after amplification of chromosomes 3, 7, and 17 and also deletion of 9p from voided urine. Multicolour FISH has been shown to be particularly sensitive and specific for CIS.[37] One series demonstrated that eight of nine patients with a prior history of CIS and a positive multicolour FISH but negative cystoscopy were subsequently diagnosed with a CIS recurrence within 5 months.[38] This makes FISH potentially valuable in the detection of recurrences on treatment or surveillance. FISH generally outperforms cytology in this context, especially in the setting of intravesical therapy where inflammatory alterations in cytologic features can impair conventional urine cytology, but FISH needs in some cases at least 25 cells or more for evaluation.[39]

But not only biomarkers could improve the diagnostic of CIS. Photodynamic detection (PDD) can be particularly helpful in the detection of CIS, with rates of detection increasing from 23% to 68% with WLC alone to 91%–97% with WLC plus PDD.[40–42] Kausch et al.[40] reported an additional detection rate of 39% for CIS in a meta-analysis of seven studies that specifically reported on CIS. This detection rate decreased to 23% if the analysis was restricted to five studies with homogeneous patient populations.

The optimal use of the UBC *Rapid* Test in daily practice still remains to be defined. In contrast to dichotomized urinary tests, its quantitative character enables risk stratification for bladder cancer to be performed based on the absolute UBC *Rapid* Test value. A positive UBC *Rapid* Test result should not inevitably lead to cystoscopy. The test might contribute not only to improved detection of bladder cancer but also to improved prediction of high-risk tumours, which has also been shown for other quantitative protein-based urinary tests.[43] One approach to objectify risk stratification including various parameters would be to develop a nomogram (including quantitative UBC *Rapid* Test, grade of haematuria, smoking status, age and gender).[44] This could be of particular interest in patients with microscopic haematuria, as the recommendations for work-up of these patients including invasive cystoscopy are discussed controversially.

Conclusion

Pathological values of UBC *Rapid* Test in urine are higher in patients with high-grade bladder cancer in comparison to low-grade tumours and the healthy control group. Sensitivity for CIS and NMI-HG tumours are very high. Thus, UBC *Rapid* Test has the potential to be more sensitive and specific urinary protein biomarker for accurate detection of high-grade patients. UBC *Rapid* Test is standardized and calibrated and thus independent of used batch of test as well as study site. UBC *Rapid* Test should be added in the diagnostics for CIS and NMI-HG tumours of the urinary bladder cancer, though cystoscopy is still an important part of monitoring of bladder cancer.

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