Preliminary Results of a Multicentre Study of the UBC Rapid Test for Detection of Urinary Bladder Cancer

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Abstract. *Background/Aim: UBC Rapid is a test detecting fragments of cytokeratins 8 and 18 in urine. These are cytokeratins frequently overexpressed in tumor cells. We present the first results of a multi-centre study using UBC Rapid in patients with bladder cancer and healthy controls. Materials and Methods: Clinical urine samples from 92 patients with tumors of the urinary bladder (45 low-grade and 47 high-grade tumors) and from 33 healthy controls were used. Urine samples were analyzed by the UBC Rapid point-of-care (POC) system and evaluated both visually and quantitatively using a concile Omega 100 POC reader. For visual evaluation, different thresholds of band intensity for considering a test as positive were applied. Sensitivities and specificities were calculated by contingency analyses. Results: We found that pathological concentrations by UBC Rapid are detectable in urine of patients with bladder cancer. The calculated diagnostic sensitivity of UBC Rapid in urine was 68.1% for high-grade, but only 46.2% for low-grade tumors. The specificity was 90.9%. The area under the curve (AUC) after receiver-operated curve (ROC) analysis was 0.733. Pathological levels of UBC Rapid in urine are higher in patients with bladder cancer in comparison to the control group ($p<0.0001$). Conclusion: UBC rapid can differentiate between patients with bladder cancer and controls. Further studies with a greater number of patients will show how valuable these results are.*

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It is known that urinary bladder cancer has a high rate of recurrence; a significant number of non-invasive tumors will progress to muscle-invasive disease. pTa tumors, the most common form of non-invasive bladder cancer, are mostly low-grade and often recur, but rarely progress to invading the lamina propria (pT1) and becoming muscle-invasive tumors (pT2-T4), whereas carcinoma *in situ* (Cis) are always high-grade and are thought to be the most common precursor of invasive tumors. Tumour grade and stage are not accurate in predicting the biological behaviour and thus guiding the choice of treatment, especially in high-risk cases (1-4).

Bladder cancer is one of the most expensive malignancies in Western countries; the cost from diagnosis to death was calculated as the fifth highest of all tumor types (5, 6). Therefore bladder cancer markers are needed to reduce cost intensity and the need for painful examinations such as cystoscopies. A definition of risk groups could help to determine which treatment is the best for the patient.

It seems that a urinary-based assay might 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. But it is unlikely that one single molecular marker can detect all bladder cancer accurately.

Monitoring of patients with non-invasive bladder cancer is necessary due to its recurrence rate and progression risk. It seems attractive to use urine based tests to detect or exclude tumor recurrence. Diagnosis and aftercare are still based on urine cytology and diagnostic cystoscopy. New markers in this field might allow for actual after-care strategies to be modified, even simplified. Cost, patient load and cost of cystoscopies in aftercare are important reasons for the use of urinary tests. 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 (7).

Cytokeratins are intermediate filaments of the cyto sceleton. The main function of cytokeratins is to enable cells to withstand mechanical stress. Twenty different cytokeratins have been identified in humans, and cytokeratins 8, 18, 19, 20 have been identified as being important in bladder cancer (8).

Cytokeratin 20 is expressed in transitional cell carcinoma by all cells, in normal urothelial cells only by the cover cells. It can be measured in higher levels of tumors. The other cytokeratins such as 8, 18, and 19 are expressed at higher levels on urothelial cells and may be elevated due to a higher cell turnover rate. Reverse transcriptase-PCR (RT-PCR) or immunocytochemistry was used to measure cytokeratin 20 in exfoliated cells. The sensitivity of cytokeratin 20 in all used methods ranges between 78% and 87%. The specificity ranges between 55% and 80% (9-11).

Fragments of cytokeratin 8 and 18 can be measured qualitatively with the UBC Rapid test. The evidence is low for low-grade tumors and benign urological diseases (12, 13). The urine soluble cytokeratins 8 and 18 can also be detected quantitatively with monoclonal antibodies using sandwich-ELISA.

The aim of the present study was to evaluate the diagnostic sensitivity and specificity of UBC Rapid in patients with urinary bladder cancer comparing with healthy individuals.

Materials and Methods

Patients. For this prospective study, 92 patients with confirmed bladder cancer and 33 healthy controls were included between January and September 2014 at the Department of Urology, HELIOS Hospital Bad Saarow (study centre I) and Lukaskrankenhaus Neuss (study centre II), Germany. The study was approved by the local Institutional Review Board of Landesärztekammer Brandenburg. All patients with confirmed bladder cancer underwent cystoscopy, bladder ultrasound, and transurethral resection of bladder tumor 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, stones in the urinary tract, other tumor diseases, diabetes mellitus, infections, and pregnancy.

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. Firstly the results of the UBC Rapid Test were evaluated visually. The presence of a test band after 10 minutes of incubation was subdivided into three categories (no band, weak band intensity, and strong band intensity). After visual evaluation, the test cartridges were analyzed by the photometric point-of-care (POC) system concile Omega 100 reader (concile GmbH, Freiburg/Breisgau, Germany) for quantitative analysis. 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. Statistical calculations were carried out with MedCalc version 12.2.1 (MedCalc Software) for ROC curve analysis. The area under the curve (AUC) ROC curves were estimated according to the method of Parker and DeLong (14). ROC curves were used to compare specificities at given sensitivities. *p*-Values of less than 0.05 (2-sided test) were considered significant.

Table I. *Number of cases at the HELIOS Hospital Bad Saarow (study Centre I) and Lukaskrankenhaus Neuss (study Centre II).*

	Study centre I	Study centre II	Total
Bladder cancer			
All	47	45	92
Low-grade	26	19	45
High-grade	21	26	47
Control group	21	11	33

Results

A total of 125 patients were included in the study, 92 with confirmed bladder cancer and 33 healthy controls with no history of bladder cancer. The median age of the study population was 73 (range=25-92) years. Out of these patients, 97 (77.6%) were men and 28 (22.4%) were women. Among the 92 patients with confirmed bladder cancer, 45 had low-grade and 47 had high-grade BCa; 71 (77.2%) had non-muscle-invasive bladder cancer (pTa and pT1 tumors), 21 (22.8%) had stage pT2-4. Carcinoma *in situ* (Cis) was detected in 10 cases (10.9%). A total of 18 (19.6%) patients had G1 tumors, 46 (50%) G2, and 28 (30.4%) had G3 tumors.

The number of patients and healthy controls are listed in Table I for study Centre I (HELIOS Hospital Bad Saarow) and study centre II (Lukaskrankenhaus Neuss). Both groups enrolled a similar number of patients in the study.

Test performance. Visual inspection of the cartridge revealed intermediate and strong test band intensity in 11 and 42 patients, respectively. In 71 samples, no band was visible.

Sensitivity was calculated as 53.3%, specificity was 90.9%. The AUC of the quantitative UBC Rapid Test using the optimal threshold obtained by ROC analysis (cut-off=9.1 μg/l) was 0.733.

ROC curve analysis is shown in Figure 1. More details about the calculation of AUC are given in Table II. After these procedures the cut-off value for UBC Rapid was set to 9.1 μg/l for this study. The statistical significance of differences in detection between patients and healthy controls was $p<0.0001$.

Discussion

Current guidelines recommend the use of urine markers only as an adjunct to cystoscopy owing to their limited accuracy (15-17). Newer tests, such as FISH and immunocytology, have

Table II. *Area under the curve (AUC)*

AUC	0.733
Standard error[a]	0.045
95% Confidence interval[b]	0.646-0.808
z Statistic	5.175
Significance level (area=0.5), *p*-value	<0.0001

[a](14); [b]Binomial exact.

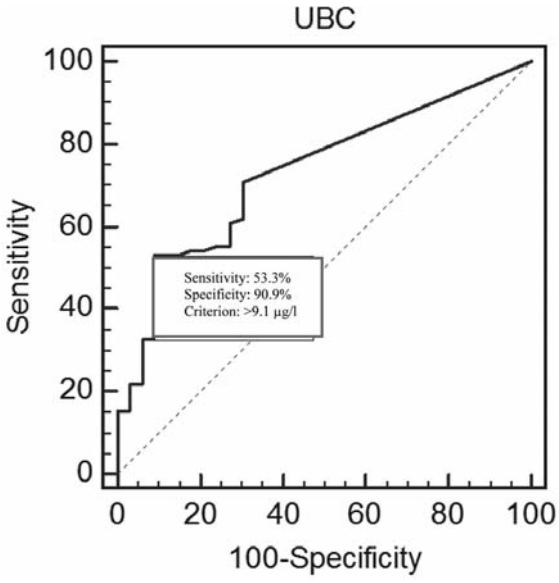

Figure 1. *ROC curve for UBC Rapid*.

shown improved sensitivity compared to cytology (2, 18, 19), 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 tumor diseases, diabetes mellitus, and the presence of stones) and the semi-quantitative evaluation process. In general, bands on lateral flow test cassettes are evaluated visually and compared with a control band. As it is not possible to determine an exact threshold for test positivity, this process leads to considerable intra observer and interobserver variability, which might contribute to the broad range of test results in prior studies (20, 21).

The aim of the present study was to evaluate the performance of a POC test for bladder cancer, which can be determined quantitatively by the use of a special POC test reader. The results of the present study show that cytokeratin concentrations determined by the POC reader significantly correlated between patients with bladder cancer and healthy controls. The AUC as a parameter of diagnostic quality of the quantitative UBC Rapid Test was calculated with 0.733 based on a cut-off value of 9.1 µg/l. The test accuracy of a manual (visual) analysis of the UBC Rapid Test strongly depended on the intensity of test bands required for a positive test. When considering test bands with strong and intermediate intensity as positive tests, the manual analysis of the UBC Rapid gave similar results to those with the quantitative determination. This might raise concerns whether quantitative analysis of UBC Rapid is really necessary to achieve good test accuracy. We also showed that the result of the UBC Rapis Test is a continuous parameter and the higher the value, the more likely is the existence of bladder cancer (13). The dichotomization of a continuous parameter leads to a significant loss of information. The semiquantitative categorization of test band intensity of POC test cassettes with different thresholds for test positivity is rarely performed. Therefore, POC tests are mostly performed as tests with dichotomized results.

Neither for UBC Rapid Test nor for other POC tests for bladder cancer (such as BTA and NMP22) do manufacturers provide protocols or images enabling adequate semiquantitative assessment. In the case of UBC Rapid Test determined quantitatively, not only did the risk for bladder cancer in general increase, but also the risk of having a high-grade tumor (G3, Cis) increased with higher test values. This feature underlines the significance of a quantitative consideration of the UBC Rapid Test, as is also the case for other quantitative urinary markers (22). A dichotomized use of this marker is not able to fully-exploit its predictive potential. When using the POC reader, interpretation of the UBC Rapid results needs to include the absolute value of the test and not only a stratification into a positive or negative result. Otherwise, there might be no additional benefit of performing the POC test quantitatively.

A study conducted by Hakenberg *et al*. showed a sensitivity and specificity of 64.4% and 63.6%, respectively, for UBC Rapid in a collective of 181 patients of which 90 had bladder cancer (23). Mian *et al*. found a sensitivity for UBC rapid of 66.0% with a specificity of 90.0% (24). However, their collective consisted of 68% patients in follow-up after transurethral resection, which might account for the difference compared with our study. Schröder *et al*. reported a sensitivity and specificity for UBC rapid of 35.6% and 75.0% (12).

The optimal use of the UBC Rapid Test in daily practice or (if implemented) in one-stop haematuria clinics remains to be defined. The test might be of particular interest for Institutions not having access to elaborate tests, such as FISH or immunocytology. In contrast to dichotomized urinary tests, its quantitative character enables risk stratification for bladder cancer to be performed based on the absolute UBC Rapid value. A positive UBC Rapid result should not inevitably lead to cystoscopy. The test results should rather be combined with clinical information (such as haematuria, age, smoking status,

and possible exogenous factors, such as infection etc.) and the result of urine cytology for optimal interpretation and clinical decision-making. Thereby, the test might not only contribute to improved detection of bladder cancer, but also to improved prediction of high-risk tumors, which has also been shown for other quantitative protein-based urinary tests (25). 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) (26). 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. As the use of cell- and protein-based tests in the screening setting has shown inconclusive results (27), we could not recommend using the UBC Rapid Test in a screening population without risk factors for bladder cancer based on these preliminary.

Conclusion

Cystoscopy is still the most important part of monitoring of bladder cancer and it cannot yet be replaced by urinary tests. However, cytology, diagnostics of haematuria and the swift tests available at a doctor's office could in combination perhaps give a chance in the future to detect this disease earlier without a high number of cystoscopies.

From this prospective, multicentre-study we can conclude from these preliminary results that UBC Rapid can differ between patients with bladder cancer patients and healthy controls. It is very important to include a higher number of samples in this study to determine how valuable these preliminary results are.

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